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Net photosynthesis of soybean leaves as influenced by anatomy, respiration, and variety

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I. INTRODUCTION

It may be said that the transformation of electromagnetic energy from the sun to chemical energy is one of the most important processes on earth. This fascinating process, photosynthesis, is responsible for synthesis of organic compounds which are necessary for life as it is seen today. The reduction of carbon dioxide during photosynthesis is accompanied by an oxidation of carbon to carbon dioxide, respiration. Net carbon dioxide exchange of autotrophic plants determines their potential yield.

Increasing economic yield of soybeans (Glycine max (L.) Merrill) is long overdue. The average energy yield (grain) of soybeans is about half that of corn (Zea mays L.) (92). This higher energy yield of corn indicates that plant systems can do better than soybeans are presently doing. One important factor to consider is that soybeans are relatively high in oil and high quality protein (90), hence, they are important to man's very survival with increasing populations. Perhaps economic yield can be increased by selecting for photosynthetically efficient varieties (20, 21, 23, 24, 81, 92).

Varietal variation in net photosynthesis of soybeans has been reported (21, 23, 24, 78, 81). Dornhoff and Shibles (23) have investigated factors related to photosynthetic rates in soybeans. Both stomatal resistance and mesophyll resistance to diffusion of CO_2 in the leaf were reported different among varieties. These differences in resistances, as well as differences in density-thickness (leaf dry weight/leaf area), of

the leaves were postulated responsible for part of the variation in net photosynthesis. No evidence of variation among varieties in light respiration was reported. Density-thickness was postulated a useful selection index for net photosynthesis. It was also shown that the development stage of the plant affected the photosynthetic rate--suggesting possible increased demand by the pods of the plant for photosynthate.

At present it is not known why density-thickness is related positively to net photosynthetic rate, but a number of investigators (3, 20, 23, 36, 45, 48, 66, 82, 112) have shown the relationship in several species. Density-thickness has been postulated related to the CO_2-diffusive resistances (20, 23, 48), and cellular volume per unit leaf area (20).

In light of the above facts, the following aims of the research were developed:

1. To verify varietal variation in net photosynthesis of soybean leaves and measure its consistency among years.
2. To study the relationships among net photosynthesis, light respiration, CO_2-diffusion resistances, density-thickness, and leaf anatomy.
3. To measure seasonal trends in net photosynthesis, light respiration, CO_2-diffusion resistances, density-thickness, and leaf anatomy.

II. LITERATURE REVIEW

This literature review will be a brief survey of papers to support the three purposes of the experiment. The literature surveyed is partitioned into two sections: control of photosynthetic rate, and respiration.

A. Control of Photosynthetic Rate

One of the purposes of earlier research (22) was to determine whether soybean varieties differed in net photosynthetic rate under normal conditions. A review of the literature pertaining to genetic differences in net photosynthesis was given in the thesis (22). A more recent and more thorough review of the subject was given by Criswell (20).

The following review is approached from a biochemical viewpoint. For the discussion of factors controlling photosynthetic rate, it is assumed that plants are in their "normal" ecological environment and that the following factors are at an optimum for photosynthesis: temperature, soil moisture, soil nutrients, and leaf age. These latter factors affecting net photosynthesis in higher plants are discussed by Nevins (75) and Criswell (19). The section on control of photosynthetic rate is divided into five subsections: supply of energy, supply of reductive power, supply of substrate (CO_2), removal of product, and enzymatic control.

1. Supply of energy

The fixation and reduction of CO_2 can be summarized into three steps (86): (1) carboxylation of ribulose-1, 5-diphosphate (RDP), (2) reduction of the two moles of phosphoglyceric acid and condensation to C_6-sugars, and (3) regeneration of the acceptor molecule (ribulose-1, 5-diphosphate). Energy is required for the process of photosynthesis. For convenience, the discussion of energy supply for photosynthesis is separated into three subjects: (a) energy required, (b) photophosphorylation, and (c) chloroplasts.

a. Energy required Photosynthesis occurs in the soybean via the reductive pentose phosphate pathway (Calvin-Benson-Bassham cycle) (44). Two moles of ATP and two moles of NADPH + H^+ are required to reduce two moles of PGA (fix one mole CO_2) (86). One mole of ATP is required to produce the CO_2 acceptor molecule (RDP). Thus 3 ATP's are required per CO_2 reduced. The overall reaction is as follows:

$$6\ CO_2 + 12\ NADPH + 18\ ATP^{-4} + 12\ H_2O \longleftrightarrow \text{glucose} +$$
$$12\ NADP^+ + 18\ ADP^{-3} + 18\ HPO_4^{=} + 6\ H^+$$

The above reaction has a free energy change (ΔG^*) of -138 kcal/mole under the following conditions: pH 7, 0.01 M concentrations, .05 atm of CO_2, 0.2 atm of O_2, and aqueous solution at 25°C (Metzler, David E., Ames, Iowa. Unpublished class notes. 1970). This is very favorable, thermodynamically. Hydrolysis of ATP gives $\Delta G^* = -11.3$ kcal/mole. Hence, a total of 33.9 kcal/mole of CO_2 reduced is required. Oxidation of NADPH + H^+ gives $\Delta G^* = -52.1$ kcal/mole or a requirement for photosynthesis of 104.2 kcal/mole of CO_2 reduced. For each mole of CO_2 reduced, 138 kcal

of energy are required. It is not likely that soybean varieties would differ in their energy requirement, unless they had different CO_2 reductive pathways.

b. Photophosphorylation The energy required for the reduction of CO_2 comes from ATP. ATP is produced by the phosphorylation of ADP via either an oxidative phosphorylation or a photophosphorylation process.

It has generally been accepted that photosynthesis by green plants requires 8 quanta per CO_2 molecule reduced (86). Rabinowitch and Govindjee (86) have stated that there is a degree of uncertainty in the widely-accepted assumption that the photochemical process in photosynthesis supplies both the NADPH + H^+ and all the ATP needed for the Calvin cycle. It is fairly safe to say that photophosphorylation will supply at least 2 ATP per 8 quanta absorbed (86). Most investigators, however, believe that there are two phosphorylation sites on the electron chain in plants (64). These sites will provide 4 ATP in noncyclic conditions. It is highly probable that some cyclic photophosphorylation, which would yield more ATP, occurs in plants (64).

If ATP production by photosynthesis is not sufficient for the reduction of CO_2, then possibly oxidative phosphorylation fills the gap. It is possible that a similar mechanism couples phosphorylation to the electron transport chains of respiration and photosynthesis (103). It is possible that soybean varieties differ in their amounts and components of the photosynthetic electron transport chains, and hence, have differential dependency on respiration.

c. Chloroplasts The energy for photosynthesis ultimately comes from absorption of electromagnetic radiation from the sun. For four species (bean, spinach, Swiss chard, and tobacco) the average absorptions were: 400-500 nm, 92 per cent; 500-600 nm, 71 per cent; 600-700 nm, 84 per cent (72). It was shown that Ficus, compared to the four species above, had a higher per cent absorption in the green region; higher absorption is characteristic of thicker leaves (72). If differences in leaf thickness exist among varieties of soybeans and absorption of light is limiting, then part of the photosynthetic differences among varieties might be accounted for by differential absorption of sunlight. A possible dependence of photosynthetic rate on pigment content of leaves (mainly chlorophyll a + b) has often been postulated (113). It is not only important to have ample light absorbing pigments, but a leaf should have the pigments properly oriented for energy transfer (86).

Govindjee (42) has presented an interesting diagram of lifetimes of various events ranging from light absorption to cell growth: act of light absorption, 10^{-15} sec; lifetime of second singlet excited state, 10^{-13} to 10^{-12} sec; lifetime of first singlet excited state--energy transfer, 10^{-9} to 10^{-8} sec; oxidation-reduction of chlorophyll, 10^{-9} to 10^{-4} sec; O_2 evolution, 10^{-5} to 10^{-2} sec; CO_2 assimilation, 10^{-4} to 1 sec; cell growth, 1 sec or longer. Kok (55) presented a paper on the rate-limiting reactions in photosynthesis, with emphasis on algal cultures. CO_2 supply was not limiting, since ample CO_2 or bicarbonate was provided. By the use of artificial electron acceptors (dyes), Kok was able to show that the carbon dioxide reduction pathway was not limiting. He cites

evidence from pulsating experiments that the delay between light absorption and appearance of O_2 is in the order of milliseconds. One of the rate limiting steps may be in the evolution of O_2, since two rate limiting steps have been postulated (55) and the pulsating experiments were done in weak light. Arnon et al. (1) have demonstrated tight "coupling" between electron transport and phosphorylation of isolated chloroplasts. Kok (55, p. 7) states, "The fact that under some conditions the phosphorylating site can severely limit the rate does not necessarily imply that also under uncoupled or phosphorylating conditions this very same site still sets the pace." Evidence is cited (55) that the phosphorylation site can operate very fast, and that the rate limiting step(s) are in photosystem II or a closely related component of the electron transport chain. By monitoring the fluorescence of system II pigment and bleaching of P700 (pigment absorbing chiefly at 700 nm) in saturating light, the rate limiting step was determined as the electron transport between the two photosystems (55). The rate limiting step is postulated to be between plastoquinone (?) and the photooxidant of system I (55). As a final conclusion, Kok (55) suggests the rate limiting step(s) are the transfer of electrons between plastoquinone (?) and the two electron chain components, cytochrome f and plastocyanin. Although the supply of CO_2 seems limiting in intact higher plants (discussed later), this discussion of rate limiting reactions may apply, since supply of CO_2 is probably not the sole limiting factor.

Another interesting approach to control of photosynthesis (55, 86) is to consider the maximum flash-yield of photosynthesis as a measure of

available amount of a rate-limiting enzyme. It is postulated that the ratio between the concentrations of chlorophyll and the rate limiting enzyme, in typical healthy plant cells, is about 300 to 1 (55, 86). This concept led to the postulation of a photosynthetic unit of 300 chlorophyll molecules per enzymatic center (55, 86). Rabinowitch and Govindjee (86, p. 70) state:

> In direct sunlight, a chlorophyll molecule will absorb photons at the rate of one to ten per second, while a good enzyme can easily transform 1,000 to 10,000 substrate molecules each second; it can thus keep pace with the substrate supply from several hundred chlorophyll molecules.

It also may be said that if more than one enzymatic reaction is slow, they all affect the saturation rate of the overall process, not just the slowest (86).

Density of chloroplasts (number/mm^2 leaf area) for several species are as follows: Tropaeolum majus, 3.83×10^5; Phaseolus multiflorus, 2.83×10^5; Ricinus communis, 4.95×10^5; and Helianthus annuus, 4.65×10^5 (46). Heath (46) cites a report of Ricinus communis having an average of 36 chloroplasts per palisade cell and 20 per spongy mesophyll cell. Several other reports indicate more chloroplasts in the palisade cells relative to the spongy mesophyll cells (31, 69, 77). Cells also differ in the type of chloroplasts; some which have no well-developed grana (46, 71).

Not all cells photosynthesize with the same magnitude (31). It has been shown with $^{14}CO_2$ isotopic activity studies, that more assimilation occurs in the bundle sheath parenchyma cells of maize (71). Sugar beet

demonstrated general distribution of ^{14}C in the mesophyll cells. Moss (69) demonstrated that dicotyledons, when illuminated from the abaxial surface of the leaf, have a higher saturation illumination for net photosynthesis. He explained the results by assuming that the abaxial illumination was being absorbed more by the non-chlorophyll structures than in adaxial illumination.

In short, chloroplasts supply the entire photosynthetic apparatus. It would seem that under saturating illumination the photosynthetic rate of higher plants would be proportional to the chlorophyll concentration. But Wolf (113) has found no correlation between chlorophyll (a + b) concentration and net photosynthesis of soybean mutants. His results must be questioned, however, because the dark green plants had very low rates of net photosynthesis. Generally, chlorophyll concentration does not seem related to genotypic differences in net photosynthesis (20, 22, 46, 107). The main limiting factors which prevent a positive correlation between net photosynthesis and chlorophyll content of the leaves (per unit area) are as follows (91): (a) irradiation during the growth of the plants and (b) irradiation during measurement of photosynthesis. It would seem that some other factors controlling photosynthetic rate would affect this correlation as well. Little relationship exists between chloroplast diameters and photosynthetic rates of various species of higher plants (28).

Gaastra (38, p. 41) states, "The slope of the light curves at low light intensities indicates the maximum efficiency of light energy conversion." Efficiency of light energy conversion, in Gaastra's terms,

is the ratio of calories of CH_2O (carbohydrates) formed to calories incident light (400-700 nm)(39). Gaastra (39) reports similar efficiencies for wheat, barley, bean, grass, and kale--12.5%. He suggests, therefore, that the actual capacities of the photochemical process were nearly the same as the optimum capacity for the process. Dornhoff (22) showed a similar efficiency for soybeans. Lower efficiency (5%), however, has been reported for cotton at atmospheric CO_2 levels (7). Hesketh (47) reports similar efficiency for four genera: maple, oak, orchardgrass, and maize. Thus, it has been reported that efficiency of the light energy conversion process is probably not limiting maximum photosynthesis of a variety (20, 22).

If one accepts the photosynthetic unit hypothesis, then other arguments concerning rate limiting processes of photosynthesis may be presented. Assuming constant chlorophyll concentration of the photosynthetic units, the number of photosynthetic units per unit leaf area will be proportional to the amount of chlorophyll per unit leaf area. If the photosynthetic units (quantosomes ?) contain the entire photosynthetic system, the photosynthetic rate per unit leaf area should be proportional to the chlorophyll concentration per unit leaf area. Since photosynthesis is generally not proportional to amount of chlorophyll, something must be limiting outside of the photosynthetic units (supply of CO_2, growth regulators, etc.) or the photosynthetic units are not sufficient in themselves. The activity of the enzymatic system within the photosynthetic unit may not be proportional to the chlorophyll concentration. Another limitation may be that the energy transfer within the pigment molecules is not of the same efficiency (spatial relationship, etc.).

Rate of photosynthesis may be determined by a single, limiting factor such as, supply of reactants, temperature, or irradiance, as suggested by Blackman, as cited by Rabinowitch (85). The rate of photosynthesis increases in magnitude with the increase in any one of these factors, (F_1), as long as this factor is the "slowest". The rate of photosynthesis ceases to be dependent on F_1 when one of the other factors (F_2, F_3, F_4...) becomes limiting (85). On a photosynthesis versus a limiting factor curve, Blackman considered photosynthesis as a linear, ascending part with a horizontal plateau. He also stated that the rate of photosynthesis was assumed proportional to the one factor that is limiting under the given conditions, and entirely independent of all the other factors. Bose, as cited by Rabinowitch (85), suggested that the effect of a certain factor, F_1, on photosynthesis, is independent of the prevailing values of all the other factors, F_2, F_3, etc. Another type of kinetic curve is characterized by initial divergence from linearity, but final convergence into a common saturation plateau (85). Some researchers have concluded that the rate of photosynthesis may be dependent on several factors at the same time; that is, that when one factor ceases to be limiting, the influence of another factor increases (85). Romell, as quoted by Rabinowitch (85, p. 863), states "Blackman's term 'slowest factor' is meaningless, and that one can only speak of a slowest process in a sequence of processes." Rabinowitch (85, p. 863) states:

> Whenever "Blackman's behavior" is observed in practice, it can be assumed that one is dealing with a series of consecutive reactions that includes (at least) one step of limited maximum efficiency. The rate of the over-all process then cannot exceed the maximum rate of passage of the system through this "bottleneck".

From the above discussion, the following conclusion of photosynthesis versus irradiance is supported. Given variety A and variety B, the initial slope of the photosynthesis versus irradiance curve is the same, but the light saturated photosynthetic rates are different. Varietal differences in the efficiency of light reactions probably are not limiting at high irradiance. This does not exclude the excellent point raised by Kok (55) that one of the limiting reactions could be a dark reaction between the postulated two photosystems. Other factors in this outline may also contribute to the differences between A and B.

2. Supply of reductive power

For photosynthesis to proceed continuously, a supply of electrons (reductant) must be present. This supply of electrons ultimately comes from water in higher plants (85).

a. Reductant required As stated earlier, the Calvin-Benson-Bassham pathway for CO_2 reduction requires four electrons per CO_2 molecule reduced (86). The four electrons reduce two molecules of PGA to two molecules of triose phosphates (86). These four electrons come from the photooxidation of 2 molecules of water. The abundance of water in all cells leads one to postulate that water itself is not limiting, but this does not mean that other water-involved steps, e.g., hydration of a "water acceptor", could not be limiting (85). Nobel prize winner Dr. Calvin (15, p. 4) states, "The oxygen thing, on the other hand, is really quite mysterious. No one has any very significant ideas as to how two oxygen

atoms of water get together to make O_2." As mentioned earlier, Kok (55) cites evidence that the evolution of O_2 may be a rate limiting step in algal photosynthesis.

b. Mechanism of reduction of $NADP^+$ Chloroplasts have been shown to use $NADP^+$ as a "Hill oxidant" (86). It has been suggested that $NADP^+$ is reduced by reduced ferredoxin, or by the postulated photochemical oxidant of photosystem I (86). Another possibility is that reduced ferredoxin reduces the 2 moles of PGA itself (86). At the present level of knowledge, there seems to be no problem of supply of reduced $NADP^+$, according to the postulated mechanisms (64). Kok (55) has shown that, in the mechanism for algae, the limiting reaction is elsewhere.

3. Supply of substrate (CO_2)

The primary substrate for photosynthesis is CO_2, for it is used directly in the carboxylation of RDP. The atmosphere surrounding the leaf supplies the CO_2 for photosynthesis.

a. Atmospheric supply to the leaf There seems to be an abundance of evidence that net photosynthesis is a linear function of CO_2 concentration up to 320 ppm (13, 26, 30, 47, 68). All crop plants thus far studied in controlled environments have responded to CO_2 fertilization (111). Dry matter and seed yields from CO_2-fertilized crops have been reported over 50% higher than that of the same crop under normal (ca. 320 ppm) CO_2 levels (111). Mean daily net photosynthesis must have increased under the CO_2 fertilized conditions. Average daily net photosynthesis of three soybean communities was increased 72% by increasing the CO_2

concentration from 300 to 600 ppm (26). Egli et al. (26, p. 414) also state, "Under the conditions encountered in this study, apparently both the supply of CO_2 to the reaction site in the leaf and the radiant energy available to fix CO_2 were limiting AP [apparent or net photosynthesis]." An earlier study (18) with two soybean varieties under high CO_2 showed a marked increase in plant growth from CO_2 fertilization compared to the control. Cooper and Brun (18) also report a 40 to 57% increase in seed yield from CO_2 fertilization, which was primarily a result of higher number of pods per plant.

b. Transfer of CO_2 in the leaf From the above discussion, it seems obvious that CO_2 is one of the limiting steps in photosynthesis. CO_2 must be transported to the site of fixation from the atmosphere immediately surrounding the leaf. The CO_2 molecules first encounter external air resistance, r_a, to CO_2 transport (38, 93). Epidermal layer resistance, which consists of stomatal, r_s, and cuticular resistances in parallel, is next encountered (38). The final resistance to CO_2 transport is termed mesophyll cell resistance, r_m (38). The stomatal resistance term, r_s, by virtue of method of determination, usually consists also of cuticular resistance and resistance to diffusion of CO_2 through the intercellular spaces within the leaf (22). Other more complicated models of a leaf have included various "sources" and "sinks" for CO_2 (29, 63, 70, 101).

CO_2 molecules probably encounter both gaseous and aqueous phases of transport. The resistance to CO_2 diffusion in water is 10^4 times the resistance to CO_2 in air (46). Diffusibility of a substance in a certain medium can be expressed by diffusion coefficients. Fick's Law uses the

diffusion coefficient as its proportionality constant to adjust for diffusibility in different media. Rate of transfer via Fick's Law is proportional to cross-sectional area of the path and the partial pressure gradient and inversely proportional to path length. By using an analogy to Ohm's Law, one can substitute partial pressure gradient for potential and rate of diffusion from Fick's Law for current (46, 38). It is convenient to express resistance to CO_2 diffusion by the length of a tube of uniform unit cross-sectional area (46)--hence, units of $sec \cdot cm^{-1}$ (38).

With the aid of Fick's Law and Ohm's Law, the resistance to diffusion of CO_2 has been calculated for photosynthesizing leaves (46, 38). Heath (46) has calculated various resistances by anatomical and experimental data. Various evaporation studies have been used to estimate r_a (20, 22, 38, 46). Heath (46) cites work with evaporation from a circular disc in a wind tunnel to empirically derive a resistance equation. Equations derived from water loss from a wet blotter, under similar conditions as the leaf, have also been used (20, 22, 38, 46, 75). With the aid of wet blotters and multiple regression, Criswell (20) has developed an equation for prediction of r_a in oats, with area of leaf and air temperature as the parameters. Impens (49) indicates that since r_a is small compared to $r_a + r_s$ for H_2O diffusion, r_a can be evaluated sufficiently from windspeed and heat transfer theory and data.

Stomatal resistance to CO_2 diffusion, r_s, has been measured by a number of methods. Heath (46) presents equations for the estimation of r_s by the measurement of stomata size and number per unit leaf area. A

commonly used method for estimation of r_s is by measurement of leaf transpiration rate (and leaf temperature and humidity), subtracting r_a as estimated by evaporation from a similar wet blotter (38). This method also uses the Ohm's Law analogy. This method raises the question of estimating internal H_2O vapor concentration within the leaf (38, 93, 100). The H_2O vapor pressure at the internal evaporating surfaces has generally been assumed to be saturation H_2O vapor pressure at leaf temperature. Slatyer (93) estimates that if leaf water potential reaches -50 bars, relative humidity within the leaf would still be 96%; hence, this gives an error of not greater than 8% in the partial pressure gradient used in calculation of r_s. The concentration of solutes at the internal evaporating surfaces may lower the vapor pressure as well (93). Sites of evaporation within the leaf also may influence the interpretation of r_s for CO_2 transport. Slatyer (93) suggests two main sites of evaporation: (a) outer epidermal walls, and (b) walls of the exposed mesophyll cells. Since resistance to CO_2 transport is computed from H_2O transport by a ratio of their diffusion coefficients, the source of H_2O evaporation should be the same as the CO_2 absorbing surface. Heath (46) mentioned that many dicotyledonous leaves have few chloroplasts in the ordinary epidermal cells; hence, evaporation from epidermal cells may lead to some error in r_s for CO_2 conductance.

It is not known for certain exactly where the liquid-air interface is in the epidermis of a leaf (93). Slatyer (93) cites experimental evidence that cuticular transpiration increases severalfold after cuticle removal. It is, however, generally accepted that cuticular resistance (usually a

component of r_s and parallel to stomatal resistance) is very high (22, 93). The subject of liquid-air interface and cell wall permeability will be discussed later.

In general, stomatal resistance to CO_2 diffusion partially controls photosynthesis. Species with few stomata on the adaxial surface appear to have higher r_s (28, 62). Differences in r_s within a species have been reported (20, 23, 48); r_s was negatively correlated to net photosynthesis. However, there was no relationship between stomata anatomy and net photosynthesis of Lolium perenne L. leaves (109).

Mesophyll resistance, r_m, to CO_2 transport is primarily measured by the method of Gaastra (38). This method involves the simultaneous measurement of photosynthesis and transpiration. Total leaf resistance to CO_2 is measured by assuming a certain concentration of CO_2 at the site of carboxylation and measuring atmospheric CO_2 level. This enables one to arrive at a CO_2 potential gradient, and hence, use the laws of Fick and Ohm. The difficult problem with this method is knowing what value to use for the internal CO_2 concentration (22, 114). Some researchers have used zero-CO_2 and some have used the leaf CO_2 compensation concentration as the internal CO_2 concentration (10, 20, 22, 38, 75).

Mesophyll resistance always seems to be negatively correlated to net photosynthesis. An attempt has been made to separate the chemical and physical resistances of r_m (17). Physical mesophyll resistance (r_m) varied from 4.5 to 9.1 $sec \cdot cm^{-1}$ and chemical resistance (r_x) from 1.0 to 0.6 $sec \cdot cm^{-1}$ (17). If the assumptions inherent in this partitioning are

correct, the physical resistance is much larger than the chemical resistance to carboxylation. Brown (10) presents some evidence that part of r_m may be chemical resistance. In general, r_m is larger in magnitude than r_s (28).

Gaseous phase diffusion has been briefly discussed above. CO_2 transport is believed to be primarily by molecular diffusion in the r_a and r_s components (46). A small amount of thermal turbulence is possible within the leaf, but is probably not significant (46). Mesophyll resistance is believed to consist partially of aqueous phase transport. As mentioned earlier, aqueous phase diffusion is believed to be much slower. Protoplasmic streaming may aid in the transport of CO_2 in the aqueous phase (46). It is thought that the water-air interface is at the surface of the mesophyll cells and within the epidermal cell walls (93). CO_2 in water apparently gives the following species: CO_2, HCO_3^-, H^+, OH^-, and $CO_3^=$ (85). It was indicated in a recent review (20) that the formation of HCO_3^- is catalyzed by carbonic anhydrase. There is evidence that this enzyme is present in higher plants and that it is adaptive to low CO_2 concentrations. However, the substrate for ribulose-1, 5-diphosphate carboxylase is reported to be CO_2 and not HCO_3^- in the spinach leaf (84). Preiss and Kosuge (84, p. 435) state, "Whether carbonic anhydrase would play a role in CO_2 fixation in chloroplasts at present is unknown." Since CO_2 is present as CO_2 and as HCO_3^- in water, they both must be considered in diffusion. The relative concentration of CO_2 and HCO_3^- species is also pH dependent.

The resistance of the mesophyll cell walls is an intriguing problem. Both CO_2 and HCO_3^- transport must be considered. The question of active transport of CO_2 and HCO_3^- must be considered as well. Apparently the cell wall (of leaf) is not the main permeability barrier to solute movement (77). Differential permeability is probably regulated by the plasma membrane (plasmalemma). The lack of selectivity of the cell wall is probably a result of large crevices (interstices) (77). Cell walls consist primarily of cellulose. Other significant components are: lignin, pectin, and other noncellulosic polysaccarides, protein, bound and free H_2O, Ca salts of pectic acids, other cations, and silicates (77). Cellulose microfibrils are interwoven in the primary cell wall and parallel in the secondary wall (77). The crevices (77) in the wall between the fibers are usually several hundred Å across. Lignins tend to be hydrophobic; hence, they repel water from the cell walls (77). Hemicelluloses and pectin are negatively charged, and therefore, hinder the entry of anions into the plant cells (77). From size and charge, one would expect CO_2 molecules to be more readily transported through the cell wall than the anion HCO_3^-.

As a result of the large interstices in the cell wall, the movement of molecules may be largely in the aqueous phase. The diffusion coefficients across the cell wall are 1/10 to 1/100 that of diffusion in water (77). Pits occur in cells, but they usually occur in pairs between adjacent cells (31, 77). These pits would greatly reduce the resistance to molecular diffusion between cells.

Diffusion within the chloroplast is probably more rapid than through the plasma membrane or the chloroplast membrane (77). Water and CO_2 diffuse rapidly across the plasmalemma compared to ATP and other metabolites (77). It is postulated that the human red blood cell membrane contains pores of approximately 7-8 Å in diameter (94). Evidence also was cited that the selectivity of the membrane for ions may be also a result of ionic charges. The structure of membranes may be the secret to their selectivity. Korn (56) concludes that membrane structures are not known but there are several good models. Korn (56, p. 273) states:

> It is entirely consistent with all available data that different membranes may have different structures, that different portions of the same membrane may have different structures, or that the same section of membrane may exist in different states at different times.

With the aid of experiments using polarized light, low angle x-ray diffraction, and electron microscope, Weier and Benson (105, 106) postulate the subunit model in chloroplast membranes. Whatever the model is, it consists chiefly of lipid (phospholipids) and protein. The protein-protein, lipid-lipid, and protein-lipid interactions are no doubt involved in the continuity of the membrane and, hence, the pore sizes through the membranes. Charges lining the pores are also only speculative, but are a possible selectivity mechanism. As mentioned before, the charges near the pore will affect the diffusion or transport of HCO_3^- through the membrane. The transport of HCO_3^- and CO_2 may be aided by active transport through the plasmalemma and the chloroplast

membranes (96). Therefore, part of the CO_2 or HCO_3^- transport may be regulated by metabolic controls (discussed later).

Nobel (77, p. 19) states that "The rate-limiting step for movement of many molecules into and out of plant cells is diffusion through the plasmalemma." As mentioned before, the partial pressure gradient determines the rate of transfer of an uncharged molecule. This is also partially true for a charged molecule--active transport or not. The transport of a charged molecule is dependent also on the electrochemical potential gradient (88).

In summary, it appears that the physical resistances to CO_2 transfer within the leaf may affect the photosynthetic rate under field levels of CO_2. These differences in resistances to diffusion of CO_2 may account for part of the variation in varietal rates of net photosynthesis.

c. Anatomy in relation to CO_2 supply Two recent reviews (20, 22) have been written on this topic. A major portion of the research for this dissertation was done on this aspect of soybean photosynthesis. Specific findings with soybeans reported in the literature will be given later in relation to the research herein. As stated by Criswell (20), net photosynthetic rates within species are often positively correlated with either leaf thickness, leaf dry weight per unit area, or leaf fresh weight per unit area. This generalization is usually the finding, but not always (20).

Leaf density-thickness (leaf weight/unit area) has been shown correlated to net photosynthesis in many species (3, 20, 23, 36, 45, 48, 66, 82, 112). Net photosynthesis of sun and shade leaves of Acer trees

was positively correlated to thickness of leaf mesophyll (83). The net photosynthetic rate of Lolium genotypes was highest when the leaves were smaller (area) and thinner than less efficient leaves (109). Photosynthesis of Lolium genotypes also seems to be negatively related to thickness of leaves and the size of the individual cells within the leaves (108). El-Sharkawy and Hesketh (28) found leaf thickness of several species negatively correlated to net photosynthesis. A high dry weight per unit area has been postulated to be related to more internal cell surfaces per unit leaf area or a higher surface to volume ratio of cells (23). A higher surface area would obviously increase the CO_2 absorption surface, and hence, might be related to CO_2 supply to the chloroplasts. The above correlations also could mean that more surface area is related to chloroplasts being oriented toward the cell walls.

Relationship between net photosynthesis and cellular size within the leaves has been studied for several species. Lolium genotypes exhibited a negative correlation between net photosynthesis and mesophyll cell size (107, 108, 109, 110). Diameter of palisade mesophyll cells were negatively related to net photosynthesis among several species (28). Net photosynthesis of five tree species was highly correlated with volume of cells in the mesophyll per unit leaf area (65). Percent volume of airspace within the leaves of several species was not related to net photosynthesis (28).

The relative amount of internal exposed surface area of the leaves has been examined in relation to net photosynthesis. Ratio of internal exposed surface area to external leaf area of several species of crop

plants was not related to net photosynthesis (28). In the same study, the ratio of internal exposed cell surface to cell volume was positively related to net photosynthesis. However, Loach (65) found that ratio of internal exposed cell surface to cell volume was not correlated to photosynthesis in five tree species. He concluded that biochemical factors rather than physical diffusion resistances limited net photosynthesis.

Differences in resistance to CO_2 diffusion could be a result of variation in number and size of stomatal pores (46). The frequency of stomata may vary from 2×10^3 to 6.6×10^4 per cm^2 leaf area (46). Dimensions of the elliptical stomata may vary from 5 to 40 μ for the long axis and 2 to 10 μ for the short axis (46). Heath (46) comments that stomata widths of 10 μ are seldom observed.

The issue of CO_2 limitation is "clouded" by the very fact that the concentration of CO_2 affects at least two processes: (a) diffusion of CO_2 from the atmosphere to the reaction site, and (2) the carboxylation of the CO_2 acceptor in the CO_2 reductive pathway (85). Rabinowitch (85) suggests three reasons for predicting CO_2 concentration as having an influence on the rate of photosynthesis: (a) partial pressure gradient of CO_2, (b) dissociation, under low partial pressure of CO_2, of the CO_2-acceptor compound, and (c) the dependence of the rate of formation of this compound on the CO_2 concentration (carboxylation). Gaastra (38, p. 62) states, "Under light saturation and at normal CO_2-concentrations, the rate of diffusion determines the rate of photosynthesis, ..." His emphasis

on CO_2 diffusion resistances as limiting have been criticized by many (50, 97, 104, 114), primarily because factors other than CO_2 are possibly and probably limiting.

4. Removal of product

There are two main products of photosynthesis--hexose-6-phosphates and O_2. It is postulated that accumulation of these products may inhibit photosynthesis by a variety of possible mechanisms. The best way to approach this problem is by first discussing the method of product removal. O_2 is probably removed by molecular diffusion to the atmosphere; hence, it is affected by similar resistances as CO_2 diffusion. Some evidence with soybeans indicates that there is considerable intercellular resistance to O_2 diffusion (73).

Glucose-6-phosphate (G-6-P) from photosynthesis probably is diffused or is transported from the chloroplasts to the site of glycolysis or pentose phosphate pathway metabolism in respiration. The remainder of the G-6-P will be translocated out of the cell. According to Richardson (87), most of the sugars are translocated as sucrose. G-6-P must, therefore, be converted to sucrose and other translocatable substances. Therefore, the synthesis of sucrose may limit photosynthesis. A modified-Munch system for translocation seems to be the only mechanism that is workable--with the present state of knowledge (6). Additional energy for the system to work may come from an active mechanism in transport (6). Translocation has been shown to depend on the metabolism of the living

cells (88). In essence, then, the removal of G-6-P from the carbon reduction cycle will depend on the general metabolic state of the leaf cells.

The literature provides researchers with a number of possible mechanisms for the depression of photosynthesis by its assimilate. Neales and Incoll (74) cite some possible mechanisms suggested by Wilson: (a) increasing the rate of respiration, (b) reducing the number of light quanta reaching the chloroplast, (c) reducing the rate of the chloroplasts reactions in which $NADPH_2$ and ATP are produced and CO_2 is elaborated into organic compounds, and (d) reducing the CO_2 concentration at the chloroplast. Beevers (4) cites several other possible mechanisms: (a) mass action, (b) distortion of the chloroplast by starch grains, and (c) increased sugar concentration inhibiting phloem loading. As the reader can now observe, there are many ideas about the subject, but no consistent accepted mechanism.

There has been a multitude of experiments designed to prove or disprove the hypothesis of "sink" regulation of photosynthetic rate. In general, most reviews of the subject conclude that there is probably some sink regulation of photosynthetic rate (4, 20, 43, 74).

5. Enzymatic control

With the rapid advance of research on biochemical control mechanisms of various pathways, it is felt that a brief treatment of the subject is in order. The supply of energy or the ratio ATP/ADP has already been discussed. The ratio of $NADPH_2$/NADP also has been presented briefly.

Product inhibition via mass action has been presented in the previous section of this literature review. Some other aspects of biochemical control covered in relation to photosynthesis are as follows: enzyme activities, supply of cofactors, allosteric inhibition and activation, feedback inhibition, and hormonal and genetic regulation.

Fortunately, the subject of regulation of photosynthesis has recently been reviewed by Preiss and Kosuge (84). The enzyme that has received the most attention is ribulose-1, 5-diphosphate carboxylase (RDPCase). RDPCase appears to be regulated by light (84). Evidently the enzyme is light-activated instead of its synthesis being light-initiated (84). Another stimulator is a light-activating factor (LAF), which has been extracted from tomato leaves with cold absolute ethanol (84). It is postulated that LAF and other factors may function as regulators. The LAF and light activation do not explain the difference in HCO_3^- K_m's (Michaelis constants) between whole chloroplasts and isolated RDPCase (0.3-0.6 mM and 20 mM, respectively). Preiss and Kosuge (84) suggest that other factors are necessary for high activity; possibly these factors are lost in isolation. It has been speculated that protein removed during isolation of RDPCase may participate in an activating or concentrating mechanism for CO_2 (84). Mg^{++} sometimes (in some species) decreases the K_m for HCO_3^- from 20 to 5.6 mM; hence, it is necessary as a cofactor.

A sigmoidal kinetic curve of RDPCase for HCO_3^- has been reported for two species of bacteria (84). This suggests that RDPCase may be regulated by a finer control mechanism (allosteric effectors). Unfortunately,

this information is difficult to interpret since the true substrate may be CO_2 and, also, the sigmoidal curve is not always observed (84). Feedback inhibition by citrate and 3-PGA has been demonstrated for RDPCase, as well as evidence for a regulatory role in Kreb's cycle, glycolysis, and gluconeogeneous (84). Evidence for a two subunit enzyme in spinach leaf gives more support to RDPCase as a regulatory enzyme (84). Repression of RDPCase has also been reported (84). Criswell (20) cites some evidence that RDPCase activity evidently is correlated to photosynthetic rate in higher plants.

Other photosynthetic enzymes are apparently regulated by light. Glyceraldehyde 3-phosphate dehydrogenase (GPDH) appears to be light activated (84). GPDH evidently consists of two forms (84)--(a) NAD dependent, and (b) NADP dependent. It has been postulated (84) that light serves to interconvert the two forms of GPDH.

Fructose diphosphatase (FDPase) has been reported to be light activated (84). A number of other factors are also required for activation (84): Mg^{++}, reduced ferredoxin, a protein, and a small molecular fraction. This enzyme also is shown to demonstrate a sigmoidal velocity versus substrate concentration curve. This, again, may imply control by allosteric effectors.

The phosphoribulokinase enzyme may control CO_2-fixation so that it occurs only when the cellular energy level is high (84). Part of the evidence for this control is exhibited by the fact that AMP inhibits ATP-dependent CO_2 fixation (84). Phosphoribulokinase demonstrates a

sigmoidal kinetic curve for substrate ATP and inhibitor 5'AMP (84). AMP also has been shown (84) to inhibit or antagonize the activation caused by NADH. AMP and NADH have both been shown to affect phosphoribulokinase (84). Hence, CO_2-fixation may be favored by high ratios of NADH/NAD^+ and ATP/(ATP + ADP + AMP). One study (54) indicates that the major rate-limiting factor in broken spinach chloroplasts is the regeneration of the CO_2 acceptor (ribulose-1, 5-diphosphate).

Ribulose-5-phosphate isomerase (84) appears to be regulated by a number of metabolites and compounds. The enzyme is reported inhibited by the following (84): citrate, AMP, ADP, ribulose-1, 5-diphosphate, and P_i (inorganic phosphate).

Earlier in this literature review, a point was made about the possibility of slow sucrose synthesis causing product inhibition of photosynthesis. Evidence indicates, however, that during photosynthesis, sucrose synthesis is very rapid (84). Recent kinetic experiments with nonphotosynthetic tissue have shown that both substrates of sucrose synthesis (fructose-6-P and UDP-glucose) exhibit sigmoidal velocity versus substrate concentration curves (84). Again one may postulate allosteric effectors regulating sucrose synthesis, rather than end-product inhibition of CO_2-fixation.

It is well known that plants have a delicate balance of hormones. Therefore, it is only reasonable to assume that they, directly or indirectly, influence every organ or cell within the plant. Hormones probably affect some control over photosynthetic rate as well (104). The mechanism

of hormonal action is not well understood, but the following possibilities exist (88): (a) transformation of nucleic acid information, (b) coenzymes for several enzymes, (c) allosteric effectors, and (d) promotion of various forms of RNA synthesis or inhibition of its breakdown. One may also postulate that they act through interaction with histones and DNA. Perhaps, they act as repressors and derepressors of gene activity. The regulation of gene activity may be very important in the regulation of enzyme synthesis for general plant metabolism. From this brief discussion, one can see the great number of possibilities for enzymatic control of photosynthetic rate.

* * * * *

In summary, it appears that there are a multitude of processes which could control photosynthetic rate. A closer approximation would be that there are, probably, several processes, reactions, etc., that limit or control photosynthetic rate, perhaps even in the field under limiting-CO_2 supply.

B. Respiration

Respiration is a process which is necessary for the supply of energy, reductive power, and metabolic intermediates for the plants various functions. The control of respiration is probably a result of a number of factors, either separate or simultaneous. For this reason and others, one would expect the magnitude of respiration in light to be different from that in the dark.

1. Dark respiration

Respiration will be defined as the evolution of CO_2. Agronomists usually measure photosynthesis as net carbon dioxide exchange of the leaves or canopy. Other researchers have measured O_2 uptake as their estimate of respiration.

The magnitude of dark respiration is important to the total carbon production of the community. Dark respiration rate is 30% of net photosynthetic rate in Wayne soybeans (52). A more comprehensive report of several species gives data that indicates dark respiration as 9.3% (range of 2.9 to 13.9%) of net photosynthesis (29). Dark respiration is important to production, but not of great importance in this study because there is evidence that respiration rate is probably different in light.

2. Light respiration

This treatment of respiration of leaves in light will be brief because the literature is filled with ideas and attempts to estimate it and there are several excellent reviews. A review in 1965 by Egle and Fock (25, p. 79) states:

> The effect of visible light on respiration of photoautotrophic plants has so far not been explained satisfactorily. A few experiments which resulted in an improvement of respiration with light are contradicted by other findings, which show an inhibition or no effect at all of light on the respiratory metabolism...

The definition of the term respiration has contributed much to the confusion (25).

Jackson and Volk (50) use the term "photorespiration" to describe all respiratory activity in the light. They (50, p. 385) also state:

> Estimates of respiratory activity of photosynthetic tissue during illumination are based on a variety of indirect methods, each of which includes at least one limiting assumption. Nevertheless, it is quite clear that substantial changes in respiratory processes occur upon illumination, and that under certain conditions the light respiratory rate may be a significant fraction of the photosynthetic rate.

Most dark respiration is believed to occur in the mitochondria, but there is some evidence for the oxidative pentose pathway in chloroplasts (50). Photorespiration primarily occurs in the peroxisomes (50).

Jackson and Volk (50) give an excellent review of the different methods of estimating photorespiration. They mention at least seven methods: (a) CO_2 release into CO_2-free air, (b) CO_2 compensation concentration, (c) postillumination CO_2 outburst and illumination CO_2 insurge, (d) relationship between apparent photosynthesis and CO_2 concentration, (e) isotopic CO_2, (f) oxygen uptake, revealed by transients, and (g) oxygen uptake revealed by labeled oxygen. All the methods involving CO_2 measurement fail to estimate the CO_2 fluxes from the mitochondria and peroxisomes to the chloroplasts (50); therefore, they all underestimate photorespiration.

Various researchers have indicated different opinions on whether dark respiration is inhibited by light. A recent opinion by Walker and Crofts (102) is that there is insufficient biochemical evidence for "dark respiration" being inhibited in the light. A somewhat different opinion

is given by Jackson and Volk (50, p. 385): "Current evidence suggests that weak illumination restricts dark respiratory processes and, at least in high compensation species, induces a light-dependent CO_2 release process..."

Walker and Crofts (102) briefly discuss possible roles for photorespiration. Evidence seems to indicate that glycolate metabolism is involved in photorespiration (50, 102). The interconversion of glycolate and glyoxylate by glycolic oxidase may be related or involved in general metabolism, stomatal opening, and noncyclic photophosphorylation (102). The metabolite, glyoxylate, may be necessary for sufficient serine biosynthesis (102). Walker and Crofts also suggest that photorespiration may be merely a wasteful process--hence, a concern to the agronomist.

III. EXPERIMENTAL PROCEDURE

Emphasis within this section will be placed mainly on methods of measurement and calculations. This section is divided into three parts: plant material, measurement of H_2O and CO_2 exchange rates, and measurement of leaf anatomy.

A. Plant Material

The plant material used in this experiment will be discussed from two aspects: selection of varieties, and culture of plant material. There seems to be sufficient evidence that the quality of the plant material is of prime importance in photosynthesis research (22).

1. Selection of varieties

The selection of soybean varieties (Glycine max (L.) Merr.) was based on results of 1968 research (22). Only four varieties were chosen for study in 1969 because of the extremely time-consuming anatomical observations. Two high and two low photosynthesizing varieties were chosen. Another important factor to consider was leaf area, since leaf area may affect the measurement of net photosynthesis. Based on these criteria, Corsoy and Amsoy were selected to represent high photosynthetic rate varieties, and Hawkeye and Richland to represent low photosynthetic rate varieties.

In the summer of 1970, six varieties of soybeans were grown. Since one of the purposes was to measure variability between years, the same four varieties as used in 1969 were grown plus two more. Two varieties

were added in 1970 because a larger sample of the soybean population was desirable, and more time was available. Provar, because of high density-thickness, and Lindarin, because it had low density-thickness, were the varieties added in 1970[1].

2. Culture of plant material

The plants, grown in pots (plastic rectangular waste-paper baskets with 11 kg soil), were raised outside to better simulate the field light and temperature environment. In 1969, the pots were placed in three north-south rows between two greenhouses. Four rows were grown in 1970, with the outside rows serving as borders. Plants on the end of the rows were not used either year. Pots were randomly distributed in 1969, but only varieties were random in 1970, because of concern about competition between varieties. The border rows were planted to Corsoy.

The soybeans were planted May 19 and May 28, in 1969 and 1970, respectively. The plants were spaced approximately 7 cm apart in the row in 1969 and 9 cm in 1970. Row width in 1970 was 40 inches and, though it was not measured, was estimated as 30 to 40 inches in 1969.

The plants were watered upon visual signs of soil dryness. On only a few instances did the plants show visual signs of wilting, and these were on high atmospheric demand days, and when evident, wilting was primarily confined to pots at the end of the rows.

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[1]The writer is indebted to Phillip E. Winborn for his assistance and data in selecting Provar and Lindarin varieties.

The same amount of fertilizer was applied both years to the 2:1:1 (v/v/v) of soil, sand, and peat mixture. To alleviate any P and K deficiencies, approximately 100 ppm by weight of P and K were mixed in the soil mixture as $Ca(H_2PO_4)_2$ and K_2SO_4. Nitrogen was added every two weeks to the pots, beginning on June 12, 1969 and June 29, 1970 and continuing to the end of the experiment. Five applications of 50 ppm N each, or a total of 250 ppm of N in the form of NH_4NO_3, were applied. The first application of 50 ppm N was mixed with the soil before planting in 1970. What was believed to be iron deficiency appeared on July 4, 1970. Sequestrene T38 Fe iron chelate was applied on July 6 to alleviate the deficiency. Two days later the chlorotic areas were beginning to show chlorophyll synthesis.

Pest control was necessary to maintain healthy soybeans. The following pesticides applied to the foliage during the season were: malathion, chlorobenzilate, DDT, and Ortho Sevin.

B. Measurement of H_2O and CO_2 Exchange Rates

The most important measurement in this study is net photosynthesis or net CO_2 exchange of the leaves. Transpiration, leaf temperature, and air temperature are measured simultaneously with net CO_2 exchange. The measurement of these latter variables allows for estimation of diffusion resistances. This section is partitioned into three subsections: (a) basic design of the system, (b) calculations, and (c) response of leaves.

1. Basic design of the system

In order to measure varietal difference in net photosynthesis, it is convenient or perhaps necessary to measure CO_2 and H_2O exchange rates under controlled environmental conditions. To achieve controlled environment during testing, the pots containing the plants to be measured, were brought into the laboratory for testing. The terminal leaflet of the youngest fully-expanded leaf was quickly placed in the leaf chamber.

a. General operation of apparatus The apparatus is discussed in detail in a M.S. thesis by Dornhoff (22) and a Ph.D. thesis by Criswell (20). In essence, the gas circuit is an "open system" where atmospheric air is pumped through solutions to adjust the CO_2 and H_2O concentrations in the air, and then the air is forced through the leaf chamber and is exhausted. A light source is present to provide sufficient radiant energy to saturate the leaves for the photosynthetic process. A leaf chamber is provided to give reasonable control over the environment near the leaf. Temperature is controlled by an air-conditioned laboratory, a water-cooled leaf chamber, and a constant temperature water bath.

Air is sampled before and after (influx and efflux) the leaf chamber for CO_2 and H_2O concentrations. Analyzed air is exhausted. A sufficient air flow rate enables measurement of both net photosynthesis and transpiration, simultaneously.

Approximately 15 minutes per leaf are required for essential steady state rate of net photosynthesis (22). To facilitate more rapid measurement of photosynthesis, etc., two leaf chambers were used simultaneously.

While material in one chamber was being analyzed, the other chamber was used for insertion and equilibration of material with the chamber conditions.

b. Light source and flux density The light source is the same, 300-Watt reflector-flood, incandescent lamps as that reported earlier (22). In 1969, the average light flux density was approximately 7950 ft-c as measured by a Weston foot-candle meter, model 756. A radiant energy spectrum was not measured by a spectroradiometer in 1969, but was in 1968. This provided a means of estimating the radiant energy between 400-700 nm wavelengths which encompasses the range of response of photochemical pigments related to photosynthesis. The light flux density of 7950 ft-c was approximately equivalent to 34×10^4 ergs/sec cm^2 from 400 to 700 nm. This was essentially the same light flux density as reported earlier (22).

Because of the high infrared load on the leaf in the chamber compared to the sun, in 1970 $CuSO_4$ was added to the water baths between the lights and the leaf chambers. The water baths were 6.6 cm deep with 0.01 M $CuSO_4 \cdot 5\ H_2O$ solution. The addition of $CuSO_4$ considerably reduced the heat load on the leaf. The mean light flux density 25 cm from the face of the bulbs (leaf position) was 8150 ft-c via the Weston foot-candle meter and 26×10^4 ergs/sec cm^2 from 400 to 700 nm. Light flux density was measured with the ISCO model SRR spectroradiometer in 1968 and 1970, but a different instrument was used each year. Figure 1 shows the energy spectrum for the sun, 1968 conditions, and 1970 conditions at leaf level within the chamber (estimated by a simulated water jacket).

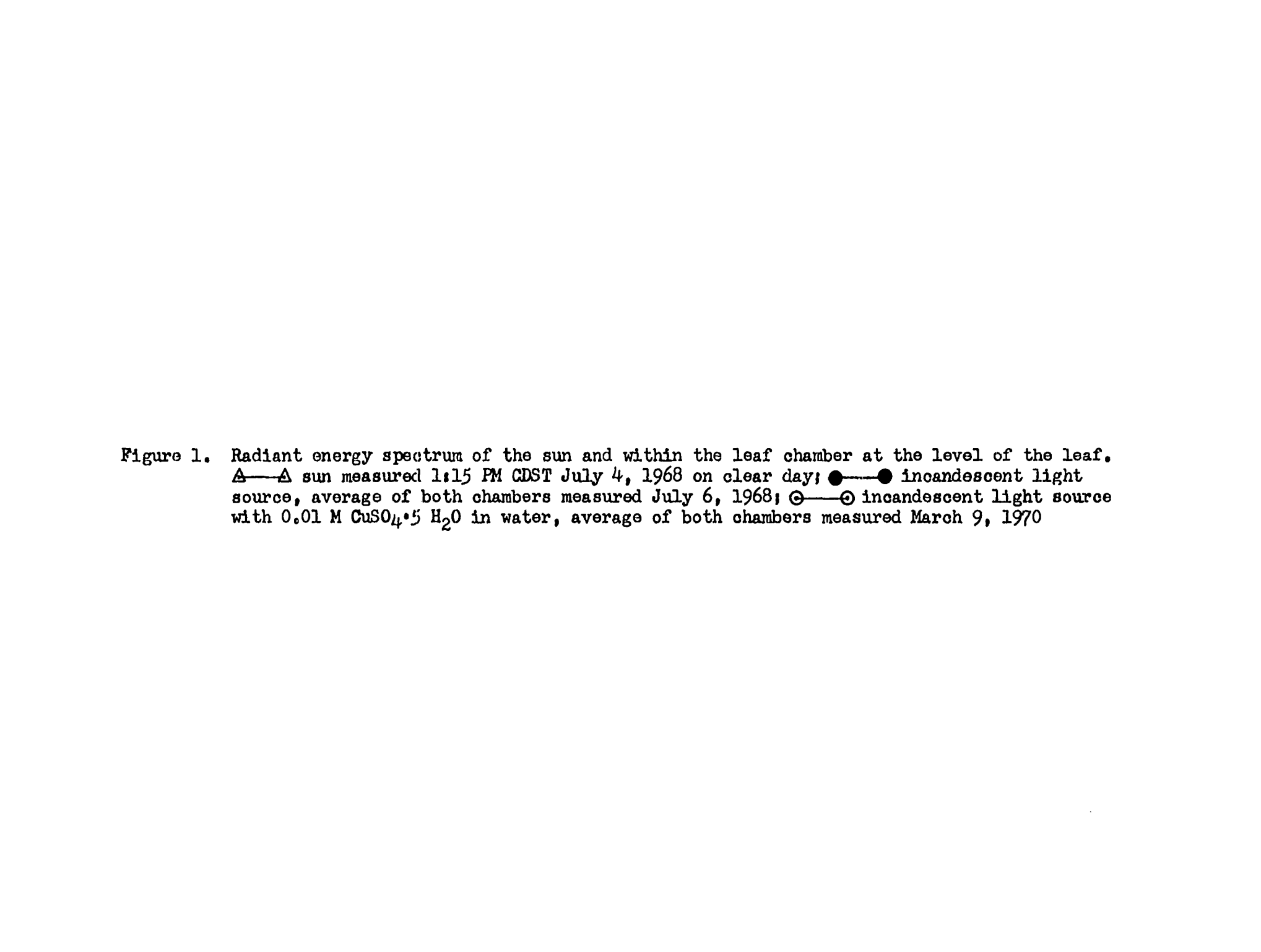
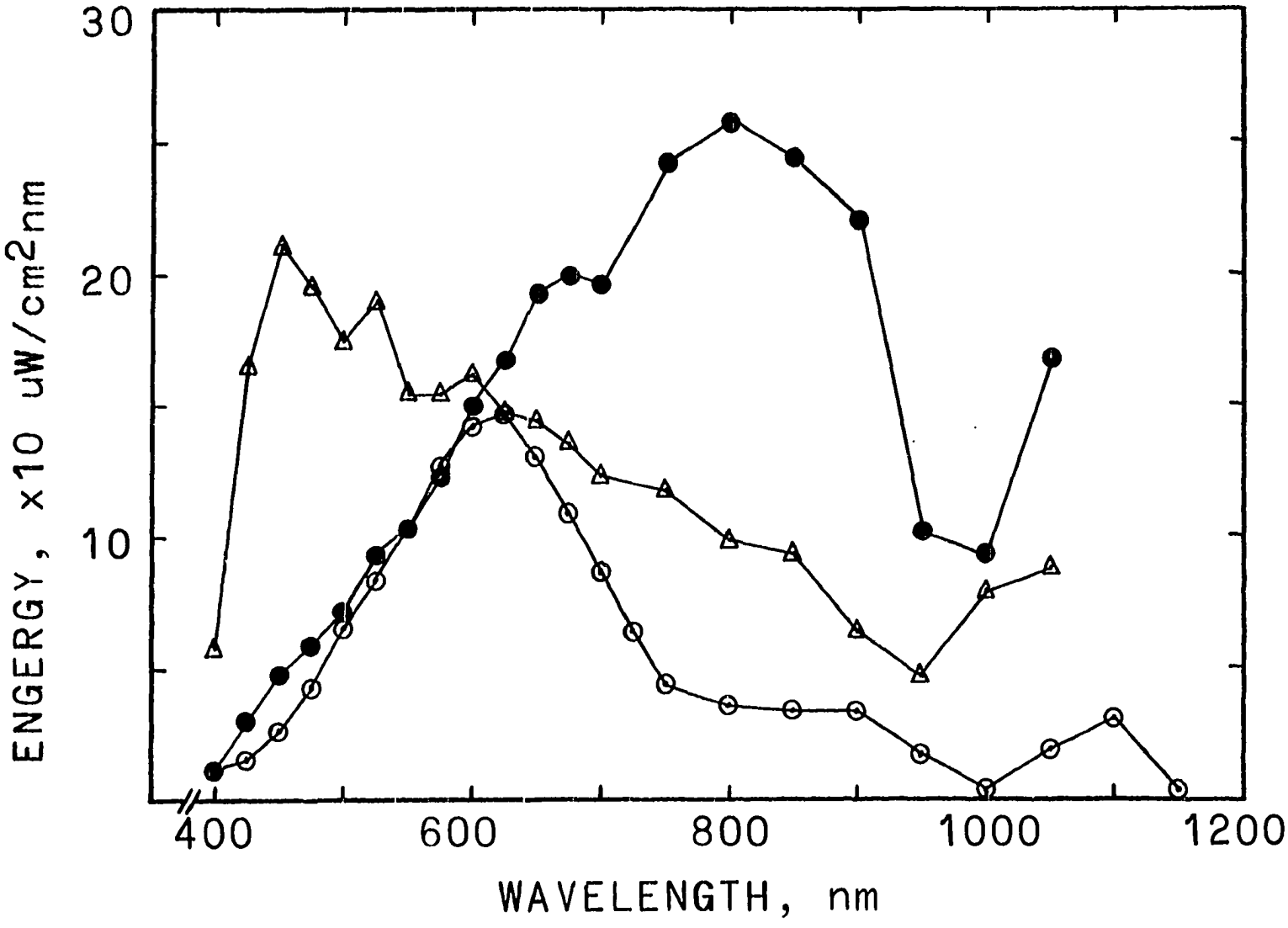


Figure 1. Radiant energy spectrum of the sun and within the leaf chamber at the level of the leaf. △——△ sun measured 1:15 PM CDST July 4, 1968 on clear day; ●——● incandescent light source, average of both chambers measured July 6, 1968; ⊙——⊙ incandescent light source with 0.01 M $CuSO_4 \cdot 5\ H_2O$ in water, average of both chambers measured March 9, 1970


30
20
10
ENGERGY, x10 uW/cm2nm
400
600
800
1000
1200
WAVELENGTH, nm

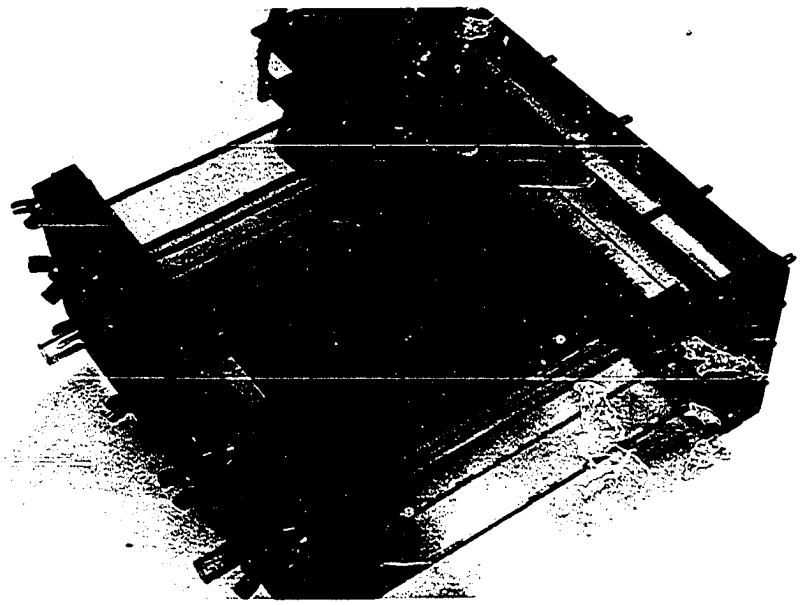
c. Supply of air to leaf chambers The gas circuit is essentially the same as used by Gaastra (38). It is the same as reported by Criswell (20). Atmospheric air is bubbled through a 6N KOH solution to remove essentially all the CO_2. CO_2 is then added back to the CO_2-free air, by a fine capillary tube, to achieve the desired, constant concentration. After the CO_2 concentration is adjusted, the humidity is adjusted by bubbling air in water at a constant temperature. Temperature of the humidified air, then is adjusted to leaf-testing conditions by pumping the air through a copper coil submerged in a constant temperature bath. Air flow rate was measured just prior to the leaf chamber by Matheson 620 BBV flow meters. Air pumps were Gast oil-lubricated, rotary-vane pumps.

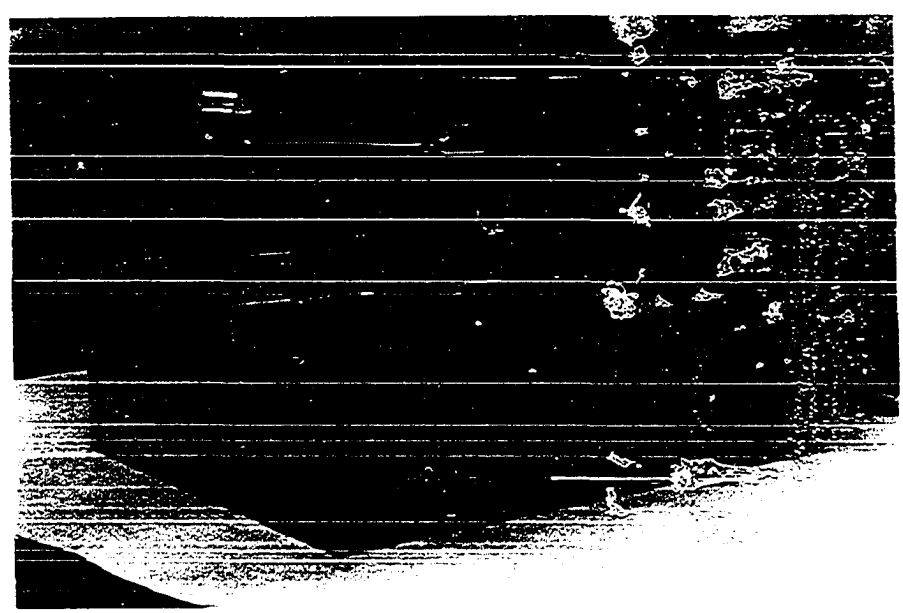
d. Leaf chambers One variable which was not held constant over the three years of testing was the leaf chambers. A different leaf chamber was used each year. The leaf chamber used in 1968 (22) was essentially of the same design as that used by Nevins (75). It was a water-jacketed leaf chamber with a slot for insertion of a leaf petiolule. The leaf chamber had an inlet manifold for even air distribution across the chamber. With this chamber, air flow rate through the chamber is critical for maintaining turbulence. A flow rate of 434 l/hr was used in 1968.

In 1969, a lower flow rate was desirable for a more sensitive CO_2 exchange rate determination. For this reason, an internal radial fan was inserted into the plexiglass leaf chamber. Figure 2 shows the leaf chamber used in 1969, which is really the same chamber as 1968 except the fan was added. The inside dimensions of the chamber were 18.3 x 14.6 x 2.5 cm. The

Figure 2. Leaf chamber used in 1969 showing a piece of blotter paper in simulation of a leaf

Figure 3. 1970 chamber with blotter paper in leaf position





radial fan was constructed so it would cause wind or turbulence toward the tip of the leaf. This provided turbulence over both leaf surfaces.

A flow rate of 400 l/hr was first tested for the windspeed measurements inside the leaf chamber. It was intended to use this flow rate for the measurements in 1969, but it was found necessary to increase the flow rate to 500 l/hr because of evidence of poor turbulence. Cobalt chloride treated paper indicated uneven turbulence at 400 l/hr. Also, there existed a temperature gradient across the leaf. The mean windspeed (500 l/hr flow rate) across the chamber at the level of the leaf was 31.7 ft/min or 16.1 cm/sec. Windspeeds were measured with a Hastings air-meter, model B-22 with a directional probe, Type S-22A. The directional probe was oriented perpendicular to the wind direction from the fan, because it could only be placed within the chamber through the petiole slot; hence, actual windspeed may be greater than reported. The windspeeds were 4 times higher at the end of the chamber near the fan than at the base of the leaf. The measurements were taken without a leaf in the chamber. The average windspeed within the chamber, calculated from flow rate and cross-sectional area measurements, was 2.8 cm/sec.

The 1970 leaf chamber was constructed quite differently. Figure 3 shows the construction of the larger leaf chamber. A larger fan was placed in the end of the chamber to increase turbulence, lower leaf temperature, and to give more uniform leaf temperatures. The chamber top was held down by spring clamps (not shown in Figure 3). Ports were placed in the side of the chamber to enable the measurement of windspeed. The inside dimensions of the

plexiglass leaf chamber were 10.8 x 14.0 x 20.3 cm. A Dayton 3160 RPM, 1/250 HP electric motor with a 4" bladed fan was used to create turbulence. The petiolule was sealed in the leaf chamber with non-toxic permagun (75). The chamber lid was removed for leaf insertion instead of the end plate as in 1969.

The mean windspeed across the chamber at leaf level with the 1970 chamber was 271 ft/min or 138 cm/sec as measured by the Hastings air flow meter. Near the fan the windspeed was four times greater than the other end of the chamber. This windspeed was measured with a flow rate of 434 l/hr, the same flow rate as used in the experiment.

e. Measurement of CO_2 concentration The CO_2 concentration was measured with a Beckman 15-A infrared gas analyzer. This analyzer was converted to a differential analyzer with two flowing cells (20). By doing this, the accuracy of the measurement was probably increased (11).

The air streams to be analyzed were dried with indicating Drierite and filtered through glass wool. The flow through the cells of the analyzer was monitored with two flow meters and maintained the same for both cells. After analysis, air was exhausted.

The electrical signal from the Beckman analyzer was fed into a Leeds and Northrup Speedomax H potentiometric, strip-chart recorder. The analyzer-recorder combination was calibrated with standard gases. Readability of the strip-chart was approximately 0.5 ppm CO_2.

f. Measurement of humidity A differential psychrometer was constructed in 1968 (22). This differential psychrometer is essentially two thermocouple psychrometers mounted together in a constant temperature

water bath. However, because of inability to maintain the two bulb temperatures exactly the same, the unit was treated as two separate thermocouple psychrometers--one for each air stream.

Wet- and dry-bulb temperatures were read with a Leeds and Northrup 8690 single-range, millivolt potentiometer. This instrument gives a readability of 0.3°C or better. There was no recorder for this instrument so the temperature was monitored and recorded when the leaf was under steady state conditions.

Calibration of the psychrometers was performed many times. Flow rate versus wet-bulb depressions were run to determine what flow rate through the psychrometer was required for maximum wet-bulb depressions. To check the accuracy of the psychrometer, air was saturated by slowly bubbling it through water at constant temperatures and then pumped through the psychrometer.

Bubbling air through constant temperature water gave varying results for the three years. In 1968, the absolute humidity was generally measured as 10 to 11 μg $H_2O \cdot cm^{-3}$ air, compared to 7.5 μg $H_2O \cdot cm^{-3}$ air for the theoretical absolute humidity of the air. There were also large variations in individual determinations. As a result of this large error, the thermocouples were reconstructed in 1969[1] with different wicking material (mercerized cotton thread). The measured absolute humidity was 8 to 9 μg $H_2O \cdot cm^{-3}$ air and the theoretical humidity was 8.5 μg $H_2O \cdot cm^{-3}$

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[1]The author is indebted to Wayne R. Hansen for help and advice in preparing more accurate wet bulbs.

air. Different wicking material, mercerized shoe laces[1], was used in 1970, because the wicks dried out occasionally in 1969. Several tests were run and the measured humidity was 9.5 to 13.5 μg $H_2O \cdot cm^{-3}$ air compared to the theoretical range of 10.5 to 12.5 μg $H_2O \cdot cm^{-3}$ air.

In 1969, the psychrometers were calibrated with different densities of sulfuric acid solutions at approximately 20°C. Generally (except at 6 mm Hg) the H_2O vapor pressure was within 2.0 mm Hg of theoretical H_2O vapor pressure over the acid solutions (range of 6 to 15 mm Hg tested). Tests were performed with H_2SO_4 solutions in 1970 and indicated that the individual psychrometers were measuring the absolute humidity of the same air within experimental error. The temperature of the acid solution was approximately 26°C, so it was not possible to compare the measured vapor pressure to theoretical vapor pressure at 20°C.

Saturated salt solutions were tried, but these were met with little success, because of lack of temperature control of solutions and long equilibrium times. The psychrometers were checked very regularly by comparing the wet-bulb depressions of the two psychrometers when no leaf was in the chamber. The results of all these tests indicated that the psychrometers require regular maintenance, and probably have significant error in absolute humidity, but the error would have had minimal effect on distinguishing varietal differences.

g. Measurement of air and leaf temperature Leaf temperatures were measured by using a spring loaded thermocouple pressed against the

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[1]Slatyer, R. O., Canberra, Australia. Wicking material. Private communication. 1970.

underside of the leaf. Two thermocouples were used for leaf temperature; each was placed approximately 1.5 cm from both sides of the midvein of the soybean leaf. Chamber air temperature was taken with a thermocouple placed approximately 1.5 cm directly below the midvein of the leaf. A thermocouple was also placed in the air stream before the flow meters to correct the volume of CO_2 for temperature. These temperatures were recorded with the same potentiometer that was used for humidity measurements.

2. Calculations

Most of the calculations used in this experiment are not new, but were reported by Criswell (20), and moreover, they are very similar to those reported by Dornhoff (22). Error analysis is important because the number of significant figures is determined by the precision of the apparatus and the number of measurements. This section is divided into three subsections: net photosynthesis, photorespiration estimates, and diffusion resistances.

a. Net photosynthesis (Pn) The conventional way to represent net carbon dioxide exchange is by net CO_2 per unit leaf area (one surface) per unit of time, mg CO_2/dm^2 hr. Measurements involved are differential CO_2 concentration across the leaf chamber, temperature of the air stream flowing through the flow meter, air flow rate, and leaf area (leaf area was determined with an optical planimeter).

Calculations of net CO_2 exchange rates involve the use of the ideal gas law. By assuming atmospheric pressure is one atmosphere, the

equation for calculation of photosynthetic rate is quite simple (Equation 1).

$$Pn = \frac{\Delta[CO_2] \cdot F \cdot k}{A \cdot T_f} \qquad (1)$$

Pn = net photosynthesis, mg CO_2 dm^{-2} hr^{-1}

$\Delta [CO_2]$ = difference in CO_2 concentration between the influx and efflux air streams to the leaf chamber, ppm (v/v)

F = air flow rate through leaf chamber, $l \cdot hr^{-1}$

A = leaf area (one surface), dm^2

T_f = temperature of air stream through flow meter, ^oK

$k = 0.536 \frac{mg\ CO_2 \cdot {}^oK}{ppm \cdot l}$

The measurements of Pn were not taken at exactly 320 ppm (v/v) CO_2 concentration for each leaf, so Pn was adjusted to 320 ppm (v/v) for varietal comparisons. By use of the slope of the CO_2 response curve for net CO_2 exchange, an equation was derived to adjust the photosynthetic rate to 320 ppm CO_2; P_{320} (Equation 2). In 1968, the CO_2 response curves were shown to be linear (no significant quadratic trends). In 1969 and 1970, only two points were used for calculation of slope of CO_2 response curve (Pn and CO_2 evolution in zero-CO_2 and light).

$$P_{320} = Pn + S(320 - [CO_2]_{OUT}) \qquad (2)$$

P_{320} = net photosynthesis adjusted to 320 ppm (v/v) atmospheric CO_2 level, mg CO_2 dm^{-2} hr^{-1}

Pn = measured net photosynthesis at chamber $[CO_2]$, mg CO_2 dm^{-2} hr^{-1}

S = slope of net CO_2 exchange of leaves vs. atmospheric CO_2 level, mg CO_2 dm^{-2} hr^{-1} ppm CO_2^{-1}

$[CO_2]_{OUT}$ = CO_2 concentration of leaf chamber efflux air stream, ppm (v/v)

Any measurement of Pn will have associated with it an error term, or a deviation from the true net photosynthetic rate. A broad frequency distribution represents a large error in measurement. The precision of the measurement is also reflected in the width of the frequency distribution (2). The best estimate of precision of an apparatus appears to be the standard diviation, σ. A more precise apparatus will have a lower inherent σ. According to Brown and Rosenberg (11), the errors in estimation of net photosynthesis are cumulative.

b. Photorespiration estimates For this research, three estimates (or indicators) of photorespiration were used: CO_2 compensation concentration, CO_2 evolution in zero-CO_2 air and light, and a calculated photorespiration resulting from a resistance adjustment of the CO_2 evolution in CO_2-free air and light. All these estimates of photorespiration have been criticized because they do not measure or take into account the flux of CO_2 within the leaf between the source (chiefly mitochondria and peroxisomes) and the sink (chloroplast) (50). Nevertheless, it was felt that some information could be gained from these variables.

CO_2 evolution in zero-CO_2 and light (R_o) was measured essentially the same as Pn (20, 22). Influx air to the chamber was essentially at zero concentration of CO_2 and the efflux air stream was above zero-CO_2 level. Hence, correction of CO_2 efflux rate to zero-CO_2 was accomplished in a similar manner as Pn adjustment (Equation 3).

$$R_o = R + S[CO_2]_{OUT} \quad (3)$$

R_o = net CO_2 evolution in zero-CO_2 and light adjusted to exactly zero-CO_2, mg CO_2 dm^{-2} hr^{-1}

R = net CO_2 evolution in near zero-CO_2 and light, mg CO_2 dm^{-2} hr^{-1}

S = slope of the CO_2 response curve, mg CO_2 dm^{-2} hr^{-1} ppm CO_2^{-1}

$[CO_2]_{OUT}$ = CO_2 concentration of leaf chamber efflux air stream, ppm

Another indicator of photorespiration, CO_2 compensation concentration, Γ, was determined as the intercept of the CO_2 response curve. By knowing the net CO_2 exchange rates at two levels of CO_2, one can determine the CO_2 compensation point concentration (Equation 4). This method of determination is based on the assumption that the CO_2 response curve within this range of CO_2 concentration is linear. Previous research indicates that the response is linear (22).

$$\Gamma = R_o/S \quad (4)$$

Γ = CO_2 compensation concentration, ppm CO_2 (v/v)

R_c = corrected net CO_2 evolution, mg CO_2 dm^{-2} hr^{-1}

S = slope of CO_2 response curve, mg CO_2 dm^{-2} hr^{-1} ppm CO_2^{-1}

R_o has been criticized as being an underestimate of photorespiration (50, 63, 89), because R_o does not take into account the flux of CO_2 within the leaf from the respiratory sites to the sites of CO_2 fixation. A correction to R_o has been applied (89). The correction involves ratios of diffusion resistances for correction of internal flux of CO_2 (Equation 5). This method of correction, however, has also been criticized (50) because it may be a minimal estimate of photorespiration as a result of R_o being measured under low CO_2 concentrations (low photosynthesis).

$$R_c = \frac{R_o(\Sigma r_\Gamma)}{r_{m\Gamma}} \qquad (5)$$

R_c = adjusted CO_2 evolution, mg CO_2 dm^{-2} hr^{-1}

R_o = corrected net CO_2 evolution, mg CO_2 dm^{-2} hr^{-1}

Σr_Γ = sum of resistances to CO_2 diffusion, assuming CO_2 concentration at the chloroplast, $[CO_2]_{chl}$, to be Γ, sec cm^{-1}

$r_{m\Gamma}$ = mesophyll resistance to CO_2 diffusion with assuming $[CO_2]_{chl} = \Gamma$, sec cm^{-1}

c. Diffusion resistances Gaastra's (38) method of measuring and calculating the diffusion resistances was used, except $[CO_2]_{chl}$ (CO_2 concentration at the site of fixation) was assumed to be Γ. Sum of resistances to CO_2 diffusion was also calculated assuming zero-CO_2 at the chloroplast, Σr (Equation 6). Total resistance to diffusion of CO_2, Σr_Γ and Σr_o are calculated from the rate of net CO_2 exchange.

$$\Sigma r_\Gamma = \frac{[CO_2]_{OUT} - [CO_2]_{chl}}{Pn(1.414 \times 10^{-6})} \qquad (6)$$

Σr_Γ = sum of resistances to diffusion of CO_2 from the external atmosphere to the chloroplast, $[CO_2]_{chl} = \Gamma$, sec cm^{-1}

Pn = net photosynthesis, mg CO_2 dm^{-2} hr^{-1}

$[CO_2]_{OUT}$ = CO_2 concentration in the efflux air, ppm

$[CO_2]_{chl}$ = CO_2 concentration at the site of fixation, ppm

1.414×10^{-6} = constant to convert Pn (mg CO_2 dm^{-2} hr^{-1}) to cm^3 $CO_2 \cdot cm^{-2}$ sec^{-1}

In order to calculate the other diffusion resistances, the measurement of H_2O exchange rates were necessary. Hence, transpiration rates were determined by measuring the difference in H_2O vapor pressure

between the influx and efflux air streams of the leaf chamber with thermocouple psychrometers (Equation 7).

$$Tr = \frac{F}{A}\left[\left[\frac{\varrho_O}{e_{SO}}\left[.5(T_{WO}-T_O) + e_{SWO}\right]\right] - \left[\frac{\varrho_I}{e_{SI}}\left[.5(T_{WI}-T_I) + e_{SWI}\right]\right]\right] \quad (7)$$

Tr = transpiration rate, mg H_2O dm^{-2} hr^{-1}

F = flow rate into chamber, $l \cdot hr^{-1}$

A = leaf area (one surface), dm^2

T_I = dry bulb temperature of influx air, °C

T_O = dry bulb temperature of efflux air, °C

T_{WI} = wet bulb temperature of influx air, °C

T_{WO} = wet bulb temperature of efflux air, °C

ϱ_I = density of water vapor in saturated air at T_I, mg $H_2O \cdot l^{-1}$

ϱ_O = density of water vapor in saturated air at T_O, mg $H_2O \cdot l^{-1}$

e_{SI} = saturated vapor pressure at T_I, mm Hg

e_{SO} = saturated vapor pressure at T_O, mm Hg

e_{SWI} = saturated vapor pressure at T_{WI}, mm Hg

e_{SWO} = saturated vapor pressure at T_{WO}, mm Hg

Calculations of laminar and stomatal diffusion resistance ($r_a + r_s$) are accomplished by a method similar to $\sum r$ except H_2O diffusion resistances must be corrected to CO_2 diffusion resistances (38). This is accomplished by multiplying the H_2O diffusion resistances by the ratio of the diffusion coefficients (D_{H_2O}/ D_{CO_2}). Gaastra (38) presents the ratio of the diffusion coefficients as 1.7194 whereas other researchers (40) give a value of 1.5636. The new diffusion coefficients, which give a

lower diffusion resistance, were used in this research. The calculation of $r_a + r_s$, is given by Equations 8, 9, and 10.

$$r_a + r_s = \frac{.563 \times 10^6}{Tr}\left[[H_2O]_S - [H_2O]_{OUT}\right] \qquad (8)$$

$r_a + r_s$ = laminar plus stomatal diffusion resistances to CO_2 diffusion, sec cm^{-1}

Tr = transpiration rate, g H_2O dm^{-2} hr^{-1}

$[H_2O]_S$ = concentration of water at the site of evaporation within the leaf, cm^3 $H_2O \cdot cm^{-3}$ air

$[H_2O]_{OUT}$ = concentration of water in the efflux air stream, cm^3 $H_2O \cdot cm^{-3}$ air

$.563 \times 10^6$ = constant to convert Tr (g H_2O dm^{-2} hr^{-1}) to cm^3 $H_2O \cdot cm^{-2}$ sec^{-1} and to convert H_2O diffusion resistances to CO_2 diffusion resistances

$$[H_2O]_S = \frac{2.89 \cdot 10^{-4} \cdot e_{SL}}{273.16 + T_L} \qquad (9)$$

T_L = leaf temperature, °C

e_{SL} = saturated vapor pressure at T_L, mm Hg

$2.89 \cdot 10^{-4}$ = constant to convert H_2O vapor pressure (mm Hg) to absolute humidity, cm^3 $H_2O \cdot cm^{-3}$ air (93)

273.16 = constant to convert °C to °K

$$[H_2O]_{OUT} = \frac{2.89 \cdot 10^{-4}}{273.16 + T_O}\left[.5(T_{WO} - T_O) + e_{SWO}\right] \qquad (10)$$

T_O = dry bulb temperature of the efflux air, °C

T_{WO} = wet bulb temperature of the efflux air, °C

e_{SWO} = saturated vapor pressure at T_{WO}, mm Hg

Laminar resistance to diffusion of CO_2 was calculated in a similar manner to Criswell (20). A series of different sized saturated blotters were used to simulate the leaf in the chamber at different air temperatures. The evaporation rate was determined from these blotters the same as transpiration from a leaf. From these evaporation rates, a regression equation was calculated using blotter area (leaf area) and chamber air temperature to predict the evaporation from a leaf with no stomatal resistance (r_s). By inserting the evaporation rate from a saturated blotter for Tr in Equation 8, r_a is determined. Inherent in this procedure is the assumption that leaf temperature equals blotter temperature and that the regression equation accurately predicts the evaporation for these given chamber conditions.

Stomatal resistance and mesophyll resistance to CO_2 diffusion were arrived at by subtraction. Stomatal resistance (r_s) is simply ($r_a + r_s$) $- r_a$. Mesophyll resistance (r_{mr}) is obtained from $\sum r_r - (r_a + r_s)$, and r_{mo} is $\sum r_o - (r_a + r_s)$.

3. Response of leaves

Recently, Nevins and Loomis (76) have expressed the importance of condition of plant material and measuring techniques for determining net photosynthesis and related variables of plant leaves. Varietal comparisons should be made under known environmental conditions. Each variety should be tested for its response to each environmental parameter thought to affect photosynthesis, or these unobserved parameters should be defined and held constant. Soil fertility and moisture content of the

soil were briefly discussed in an earlier section. These are believed to have been near optimum. No diseased plants, at least as could be determined by inspection, were tested. The response of leaves to various pesticides applied was not checked because of lack of time. Several other environmental parameters were, however, briefly examined.

a. Response to irradiance In an earlier study (22) with a limited number of leaves, it was reported that the light-response curves of net photosynthesis were different during the season. The leaves were light-saturated at approximately 12 x 10^4 ergs sec^{-1} cm^{-2} (400-700 nm) on July 9 compared to light-saturation of 30 x 10^4 ergs sec^{-1} cm^{-2} on August 21. It was postulated that the higher rate of net photosynthesis later in the season was related to the higher light-saturation.

In 1969, six light-response curves were run on each of the four varieties tested, Corsoy, Amsoy, Hawkeye, and Richland. Each one of the twenty-four light-response curves were run from high light flux density to low light by insertion of copper window screens between the light source and the leaf chamber. Figure 4 shows the mean response curve of the four varieties. An analysis of variance at each light flux density showed no varietal differences. It appears that these four varieties were light-saturated for the photosynthetic process at approximately 30 x 10^4 ergs sec^{-1} cm^{-2}. The curves were run from August 12 to August 18, 1969. This experiment also gives evidence that there is no difference in the slope of the light response at low light. The slope of the light-response curve at low light has been used to indicate the efficiency of the photochemical processes (38).

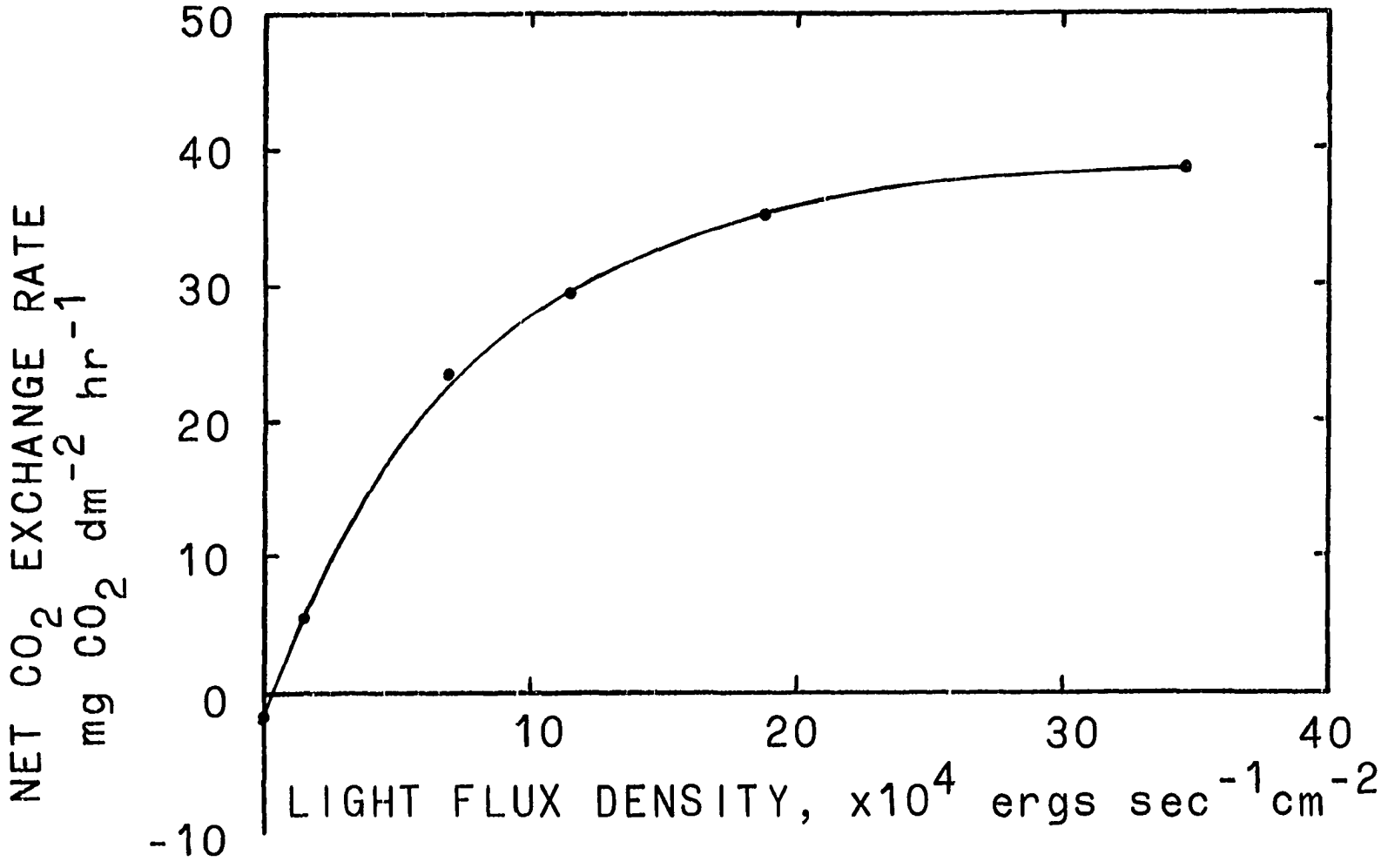


Figure 4. Light response curves of the most recent fully-expanded soybean leaves. Each data point is the mean of six measurements per variety (24 observations). Curves were performed August 12 to August 18, 1969. Experimental conditions: $[CO_2]$ = 320 ppm, VPDA (H_2O vapor pressure deficit of air) = 10 to 24 mm Hg, RH (relative humidity) = 32 to 51%, leaf temperature = 20 to 33°C

Several light-response curves were performed in 1970. The results indicate that the three leaves tested were saturated, or very nearly saturated, at the light flux density used for the 1970 variety experiments (26 ergs sec^{-1} cm^{-2}). The above results indicate that all years of research were carried out under essentially light-saturated conditions for net photosynthesis.

b. Response to humidity No tests on effect of atmospheric humidity on net photosynthesis were run in 1969 and 1970. In 1968 (22), an experiment was performed to measure net photosynthesis over a range of H_2O vapor pressure deficits. No significant effect of humidity on net photosynthesis was observed over the range of 7.5 to 11.0 mm Hg. The experiment was performed under nearly steady-state conditions.

c. Response to windspeed Design of the chamber affects the windspeed within. The chamber used in 1968 gave an average windspeed of 3.1 cm/sec. It is believed that the windspeed in the center of the chamber was greater than this, because of jet airstreams from the air inlet tube. A windspeed-response curve for net photosynthesis was performed in 1968, and an average windspeed of 2.5 cm/sec was found sufficient.

As mentioned earlier, the 1969 chambers were 1968 chambers with radial fans in them. Mean windspeed, as measured by a hot wire anemometer (Hastings), was 16.1 cm/sec. Unfortunately, the effect of the fan on net photosynthesis was not tested until the end of the 1969 measurement season. Prior to measurement of net photosynthesis, the chamber was tested by blowing smoke in the inlet tube. Smoke seemed to mix very rapidly,

and hence, it was assumed the air mixing in the chamber was sufficient. The effect of the fan was tested on four leaves (one per variety), and was found to decrease net photosynthesis by an average of 0.7 mg CO_2 dm^{-2} hr^{-1}. Another undesirable phenomenon that was noticed, was that the leaf temperature on the inlet side of the leaf chamber was much cooler than the outlet side. This difference in leaf temperature seemed greater with the fan on. Evidently, there was something wrong with the aerodynamics of the system. In essence, the fan was undesirable in the 1969 leaf chamber.

A better chamber, aerodynamically, was built in 1970. The hot wire anemometer detected average windspeed as 138 cm/sec. Windspeed was measured in direction of the propeller "wash", but in 1969 it was measured perpendicular. Hence, the difference in the detected windspeed in 1969 and 1970 may be, in part, an artifact of direction of measurement. The fan had a beneficial effect on net photosynthesis in this larger chamber (Figure 5). However, experience suggests that a fan is not necessarily beneficial in all leaf chambers.

d. Response to temperature Temperature is an obvious environmental parameter that should be investigated with any net photosynthesis study. Leaf temperature is the parameter of most interest. Research in 1968 indicated a broad optimum of between 30 and 40°C in the net photosynthesis-leaf temperature-response curves (22). These tests were not run under steady-state conditions, but were with slowly increasing temperature.

Four leaf temperature-response curves for net photosynthesis (one per variety) were performed in 1969. Again these tests were not under steady-

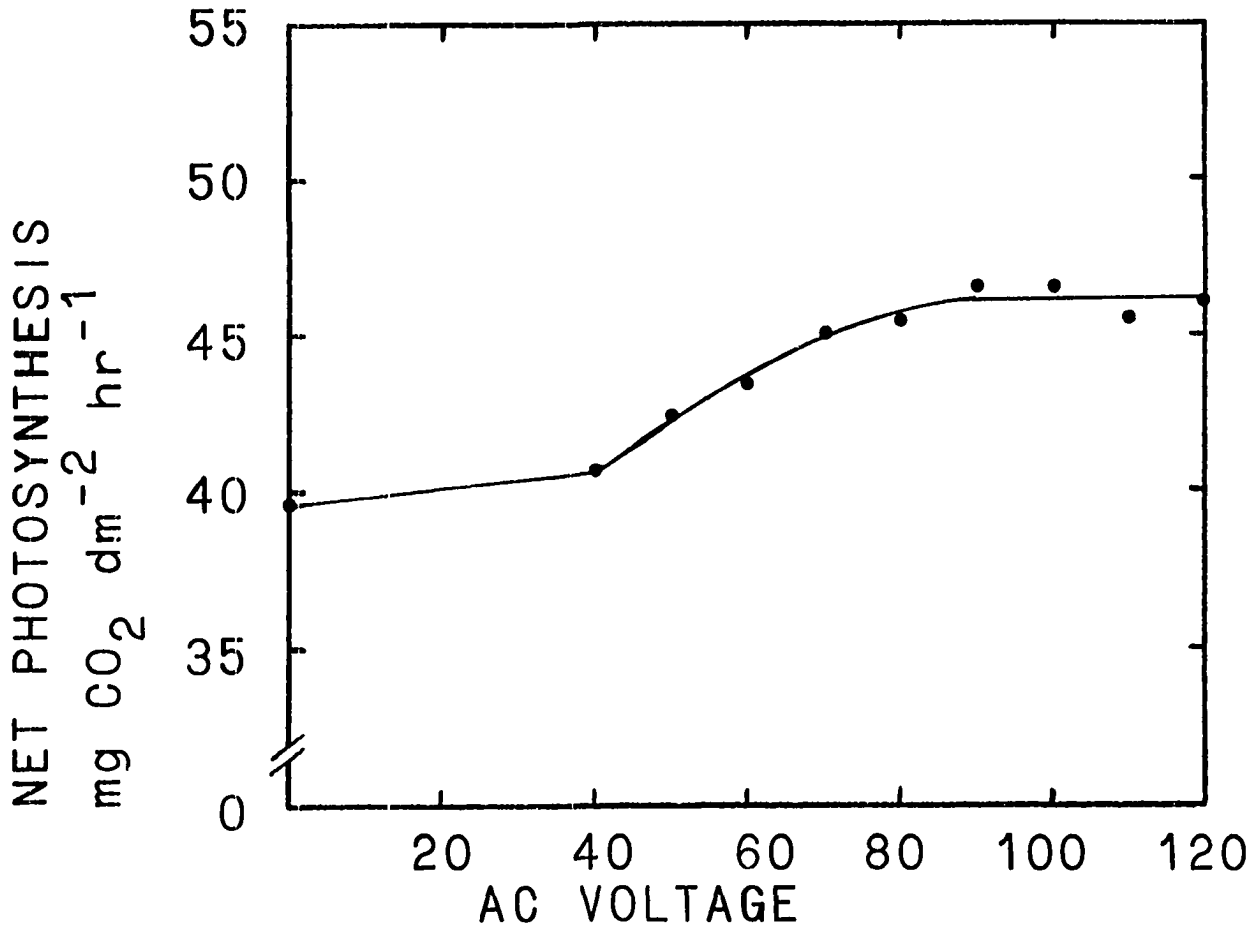


Figure 5. Windspeed-response curve for net photosynthesis of a most recent fully-expanded leaf of Corsoy on July 17, 1970. Windspeed is related to the fan AC voltage supply. Experimental conditions: Light = 8150 ft-c, VPDA = 6 mm Hg, RH = 75%, leaf temperature = 26.5°C, $[CO_2]$ = 320 ppm

state conditions, but were under slowly increasing leaf temperature. Essentially all the varieties began to decline in net photosynthesis around 35°C leaf temperature. Usefulness of these temperature curves is limited because the lowest leaf temperature used for the tests was 32°C. The curves do indicate that optimum net photosynthesis occurs below 35°C.

e. Response to leaf aging For present purposes age of the leaf will be defined as days after full lamina expansion. In 1968 (22), measurements were taken on approximately the third or fourth unrolled leaf from the top of the plants. By observation, it was believed that this represented the most recent fully-expanded leaf. A leaf was counted as number one from the top when the leaf was completely unrolled. It was assumed that net photosynthesis was optimum in the youngest fully-expanded leaf.

In 1969, an attempt was made to test the assumption that optimum net photosynthesis occurs in the youngest fully-expanded leaf. During 1969, the date at which leaves attained approximately one-half inch in length was recorded on tags attached to the leaf petiole. Six plants per variety were monitored every other day for leaf expansion. Expansion of the leaves was estimated by their maximum width. The time it took for leaves to reach full expansion was about the same for all varieties. The length of the period of expansion after tagging, however, differed during the season or stage of development of the plant. During the first two weeks in July and the first week in August the days to full expansion were approximately 12. The second two weeks in July exhibited a longer period of expansion, approximately 16 days.

Another interesting observation that came from this experiment is that varieties Amsoy, Hawkeye, and Richland ceased leaf expansion of the uppermost leaves around August 18. However, Corsoy stopped expanding around August 14. Hence, leaves tested after these dates may be affected by leaf aging. Essentially all leaves had emerged by August 6.

By tagging the leaves, it was possible to record when the leaves were fully-expanded and, hence, test them. A problem encountered was that there was, naturally, a delay in the time between when the leaves were shown fully-expanded and the testing of them. In short, there was a delay of from one to seven days between full expansion and testing of the leaves. There was more delay toward the end of the season when leaves stopped expanding.

The question of whether the third or fourth unrolled leaf from top is really the most recent fully-expanded leaf was not completely answered. However, it seems that the fourth leaf is generally fully expanded and the fifth leaf is always fully expanded.

Three small experiments were conducted in 1969 on the effect of leaf aging on net photosynthesis. Experiment 1 was a study of the effect of leaf aging on net photosynthesis of the four varieties during July 21 to 25. The leaves were essentially fully expanded on July 19. Corsoy, Amsoy, and Hawkeye were exhibiting optimum net photosynthesis from two to six days after full expansion, whereas Richland may have been still increasing in photosynthesis at six days aging.

Experiment 2 indicated the effect of degree of leaf expansion on net photosynthesis as tested from July 21 to 25. The leaves were fully

expanded approximately July 25. Corsoy, Hawkeye, and Richland appeared to be still increasing in net photosynthesis up to full expansion. Amsoy evidently was at maximum net photosynthesis at full expansion.

Experiment 3 was a longer term experiment to test the effect of leaf aging on net photosynthesis. As seen in Figures 6, 7, 8, and 9, optimum net photosynthesis does not occur exactly at full expansion, but perhaps from two to six days after full expansion. These individual curves were inserted to illustrate the reproducibility of the measurements. Each curve represents one leaf reinserted in the leaf chamber each day. This also illustrates that the measuring apparatus does not harm the leaf being tested.

C. Measurement of Leaf Anatomy

Some 1968 observations led to the research into anatomy. It was found earlier that net photosynthesis was positively correlated with leaf density-thickness (leaf dry weight/leaf area) and to stomatal conductance to CO_2 movement ($1/r_s$). Thus, the stomatal apertures, stomatal frequencies, density-thickness, and internal leaf anatomy were examined in 1969 and 1970.

Every day that net photosynthesis was measured, leaf epidermal impressions, leaf cross-sections, and leaf clearings were made for each leaf tested. Because of the large amount of time required for anatomical observations, however, only four of the 24 days in 1969 and 26 days in 1970 were analyzed. In 1969, the dates for detailed anatomical analysis were chosen on the basis of varietal differences in net photosynthesis, and

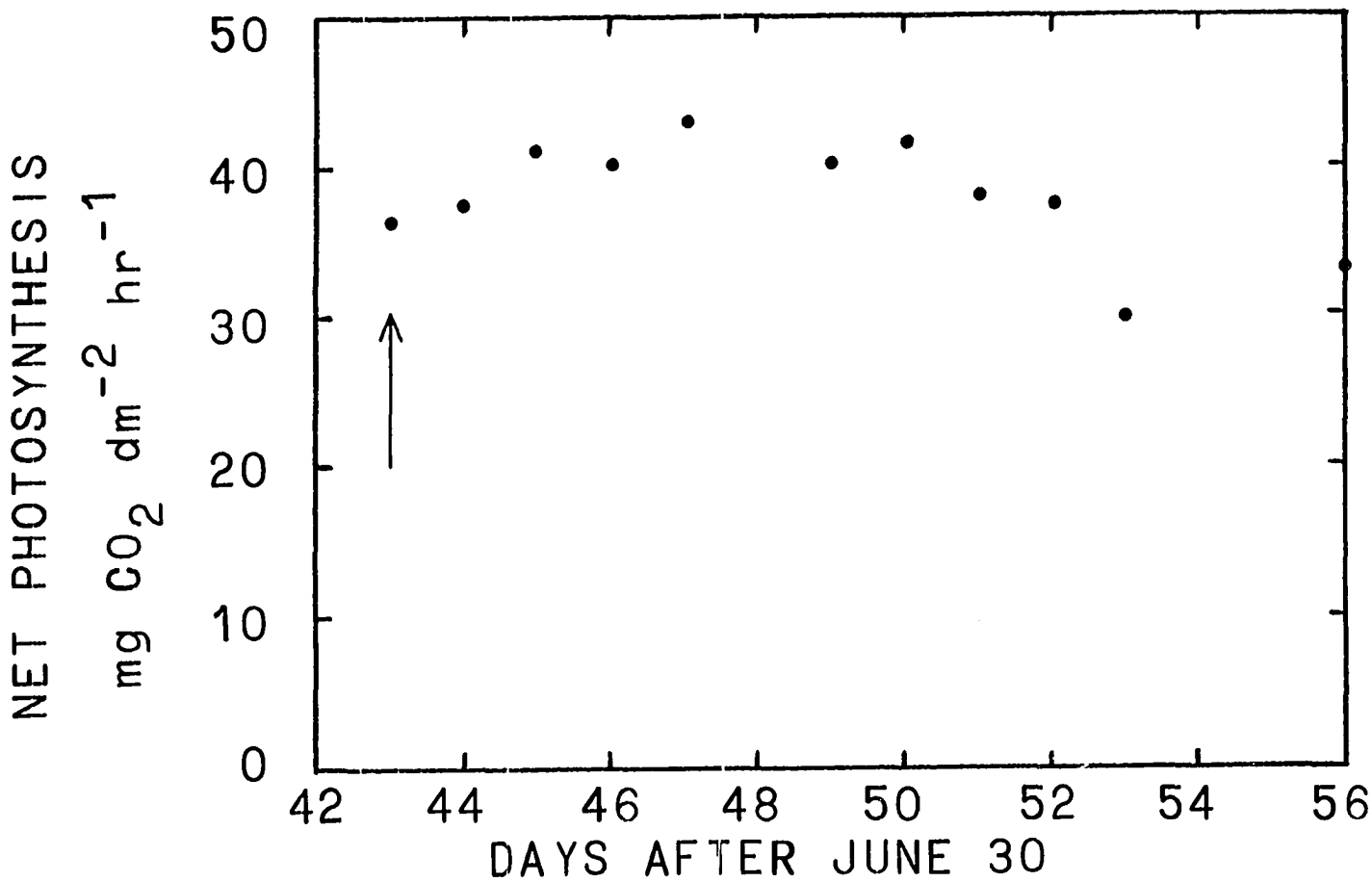


Figure 6. Effect of leaf aging on Corsoy leaf net photosynthesis, 1969. The same leaf was tested each day. Arrow indicates date of full expansion. Experimental conditions: VPDA = 22 to 46 mm Hg, RH = 34 to 23%, $[CO_2]$ = 320 ppm, light = 7950 ft-c, leaf temperature = 30.0 to 32.5°C

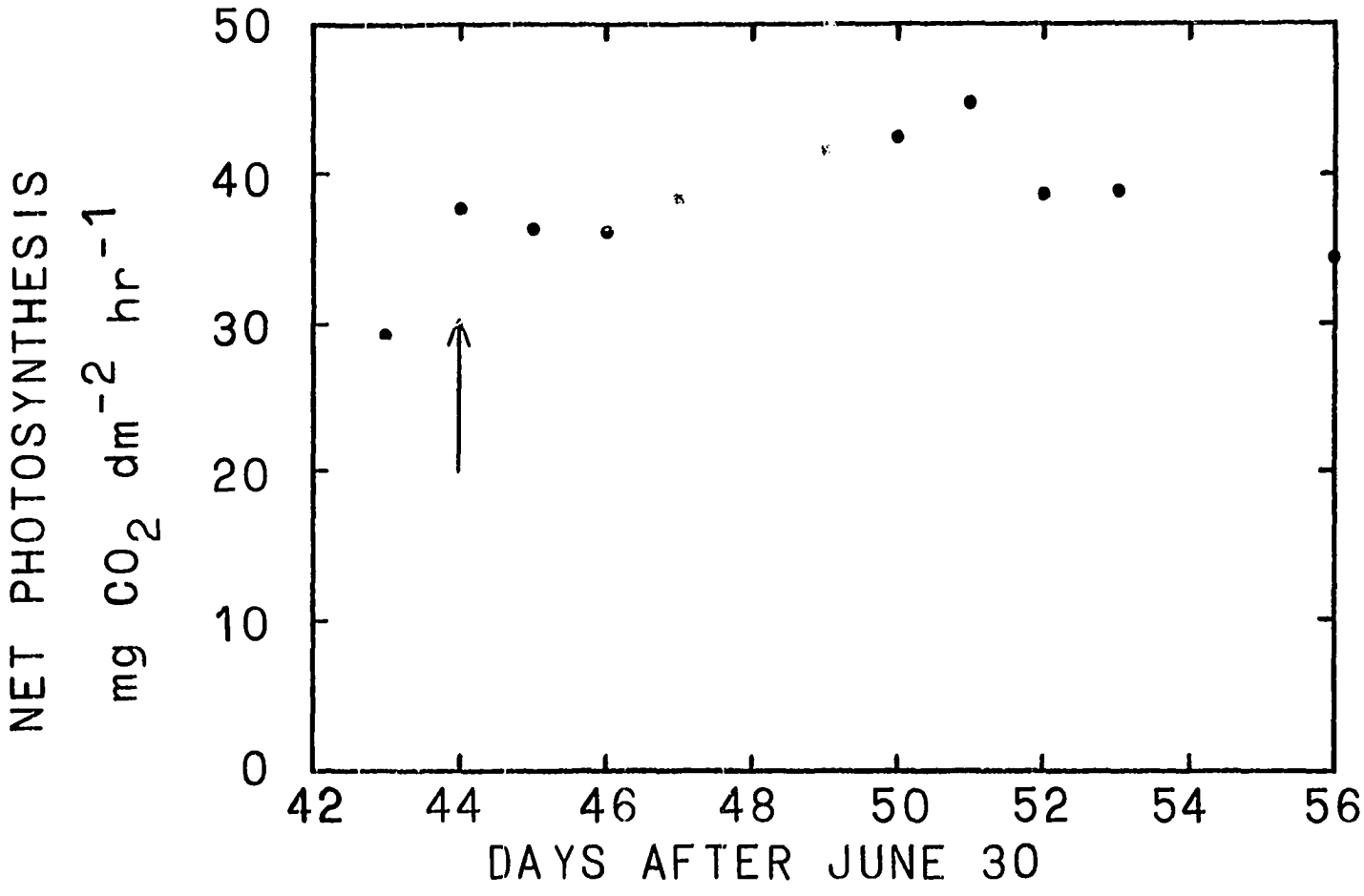


Figure 7. Effect of leaf aging on Amsoy leaf net photosynthesis, 1969. The same leaf was tested each day. Arrow indicates date of full expansion. Experimental conditions: VPDA = 25 to 41 mm Hg, RH = 38 to 31%, $[CO_2]$ = 320 ppm, light = 7950 ft-c, leaf temperature = 29.0 to 34.0°C

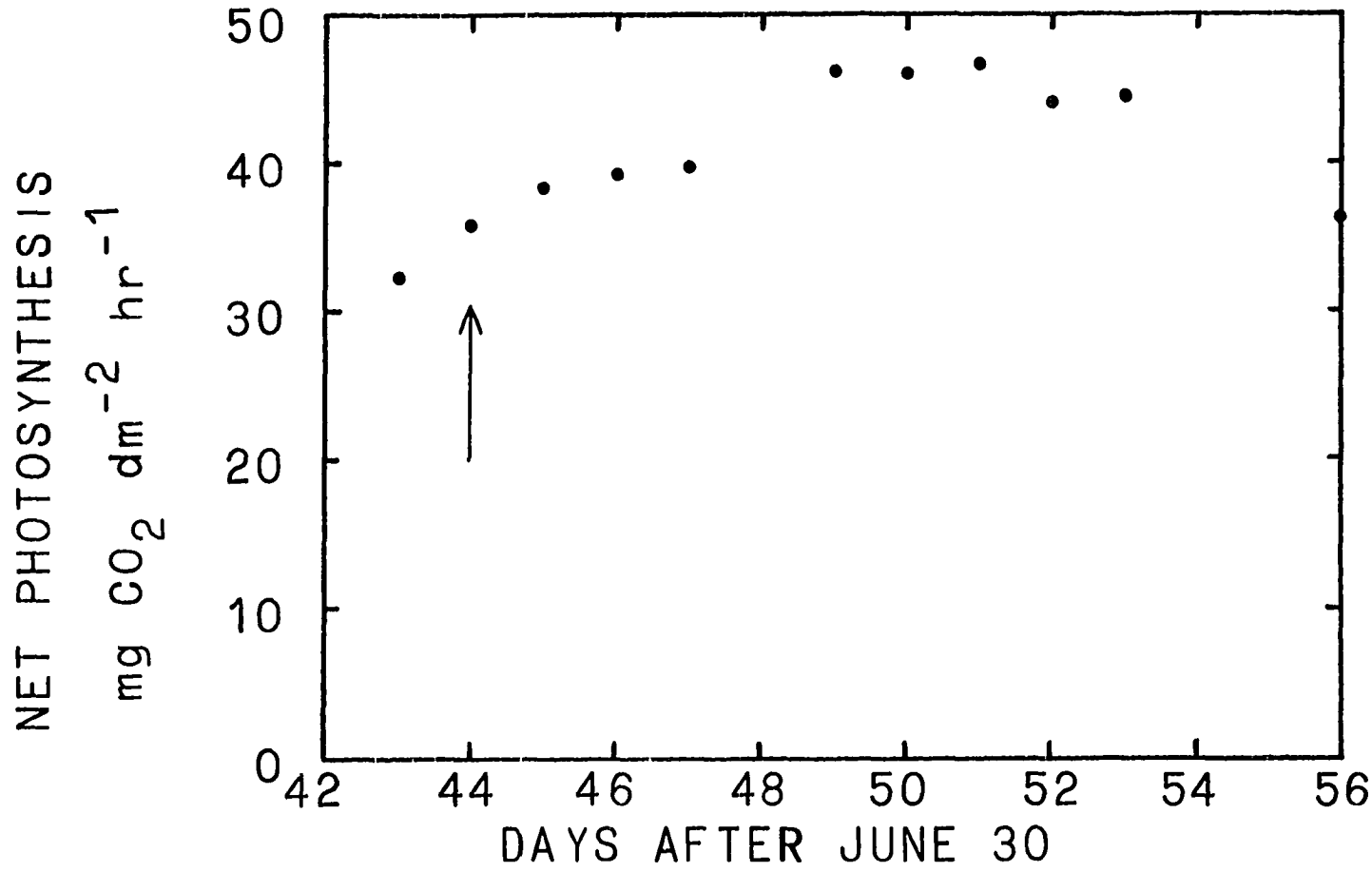


Figure 8. Effect of leaf aging on Hawkeye leaf net photosynthesis, 1969. The same leaf was tested each day. Arrow indicates date of full expansion. Experimental conditions: VPDA = 19 to 43 mm Hg, RH = 39 to 26%, $[CO_2]$ = 320 ppm, light = 7950 ft-c, leaf temperature = 29.5 to 33.0°C

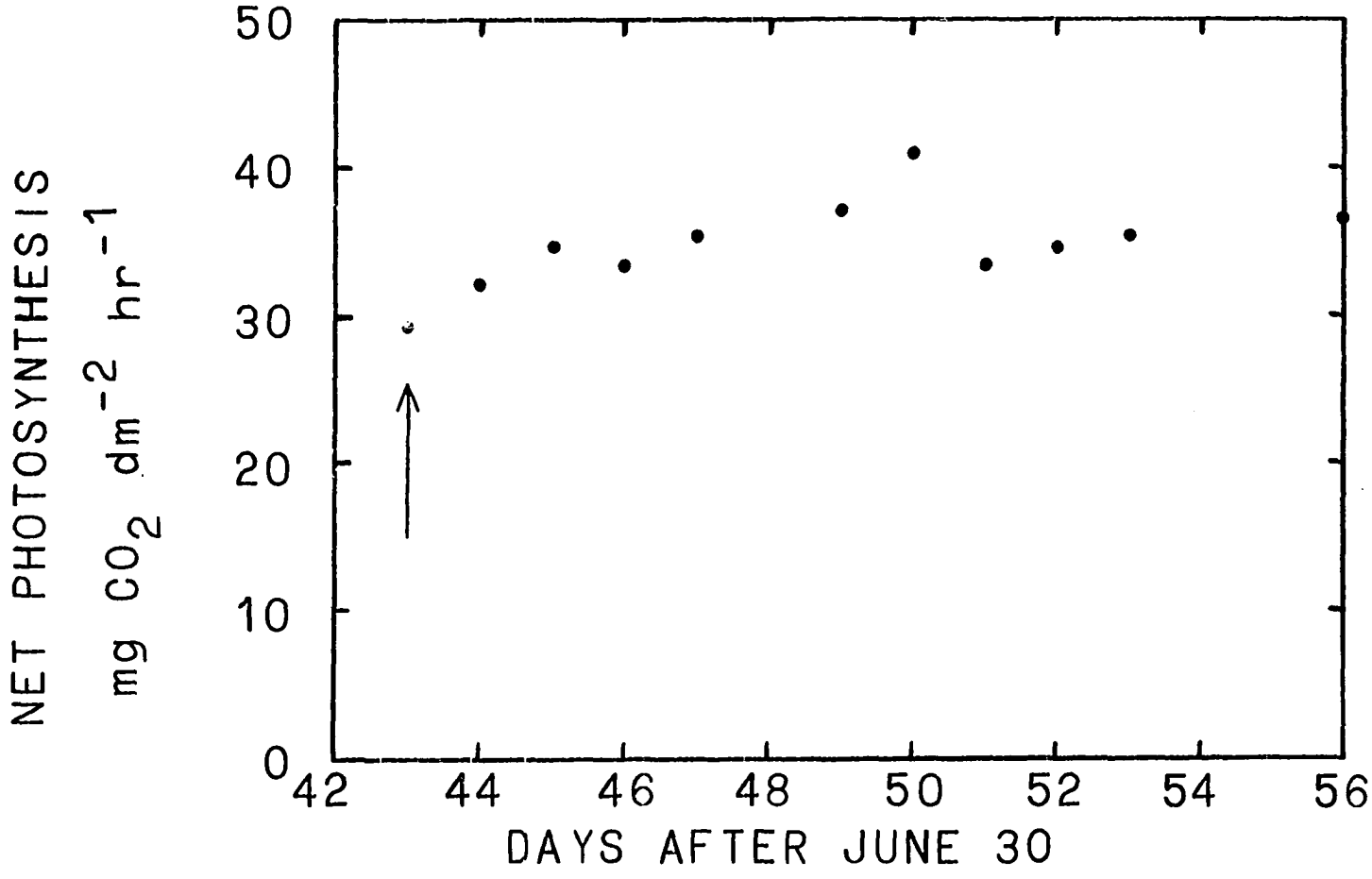


Figure 9. Effect of leaf aging on Richland leaf net photosynthesis, 1969. The same leaf was tested each day. Arrow indicates date of full expansion. Experimental conditions: VPDA = 18 to 40 mm Hg, RH = 44 to 31%, $[CO_2]$ = 320 ppm, light = 7950 ft-c, leaf temperature = 30.0 to 34.0°C

they were chosen to be approximately a week apart: July 28, August 4, August 12, and August 19. Dates for anatomical analysis in 1970 were arbitrarily chosen as every Thursday: July 23, July 30, August 6, and August 13.

1. Measurement of stomatal apertures and frequencies

Epidermal impressions of the leaf surfaces were made with silicone rubber (75, 115). The viscous silicone rubber mixture (General Electric RTV-11 and catalyst Nuocure 28) was applied to the leaves on both surfaces immediately after they were taken from the leaf chamber. Rubber was applied to the middle of the leaf lamina on either side of the midvein. It was usually dry (cured) within five minutes. After drying, impressions were peeled from the leaves and were labeled and placed in a desiccator for storage.

For a positive impression of the leaf surfaces, the silicone rubber impressions were painted with a thin film of cellulose acetate solution. The cellulose acetate solution used was a commercial, colorless nail polish. After drying, the thin film was peeled from the silicone rubber and placed on a microscope slide. The cover slip was fastened with transparent cellophane tape. The impressions of the leaf surfaces were examined under a Carl Zeiss phase microscope, model GFL 654-634. Lenses used in the microscope were as follows: Complan eyepiece KPL, 10x; Achromat phase objective, 25x; and Achromat phase objective, 40x. The microscope was set at 250x for stomatal observations. It was found that bright field illumination with a green filter and without phase contrast gave the best contrast for photomicrographs. To get better resolution for

measurements, the image was taken with a camera, Honeywell Pentax spotmatic, with a microscope adapter. Kodak panatomic-X 35 mm film with an ASA rating of 32 was used both years. Both years of anatomical research, the leaf surface impressions were enlarged to 980x for stomatal measurements. In 1969, the black and white negatives were enlarged by a film strip projector and the measurements of stomatal width, length, and densities were taken directly from the projected image. In 1970, negatives were enlarged to 980x by an enlarger on black and white kodabromide A-3 paper. Measurements were then made directly off of the enlarged picture.

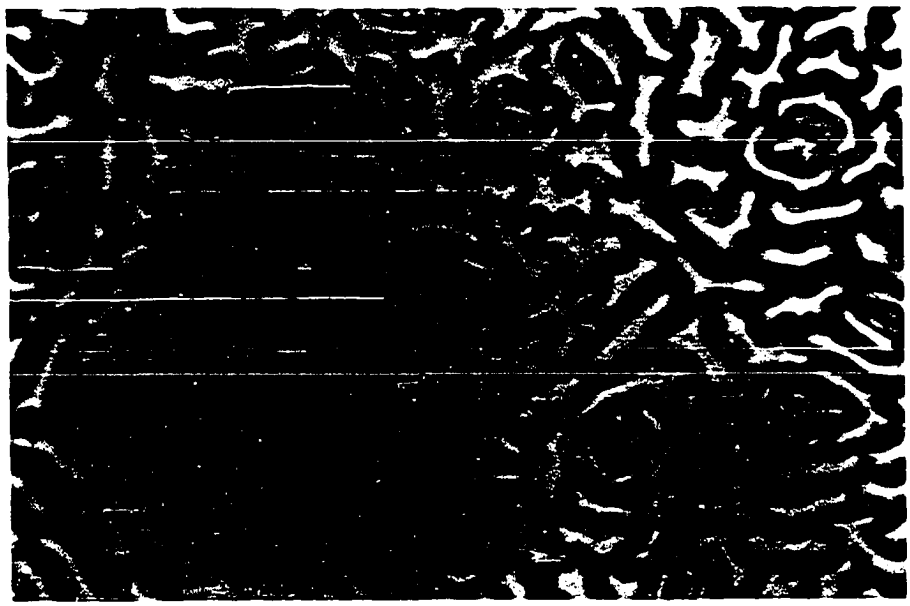
Stomatal apertures were measured as the width and length of well-defined, or in-focus stomata. Poorly-resolved stomata were not used for aperture measurements. Figures 10 and 11 show examples of the stomata impressions from the adaxial (top) and the abaxial (bottom) leaf surfaces. Degree of enlargement was determined by photographing a micrometer microscope slide through the microscope.

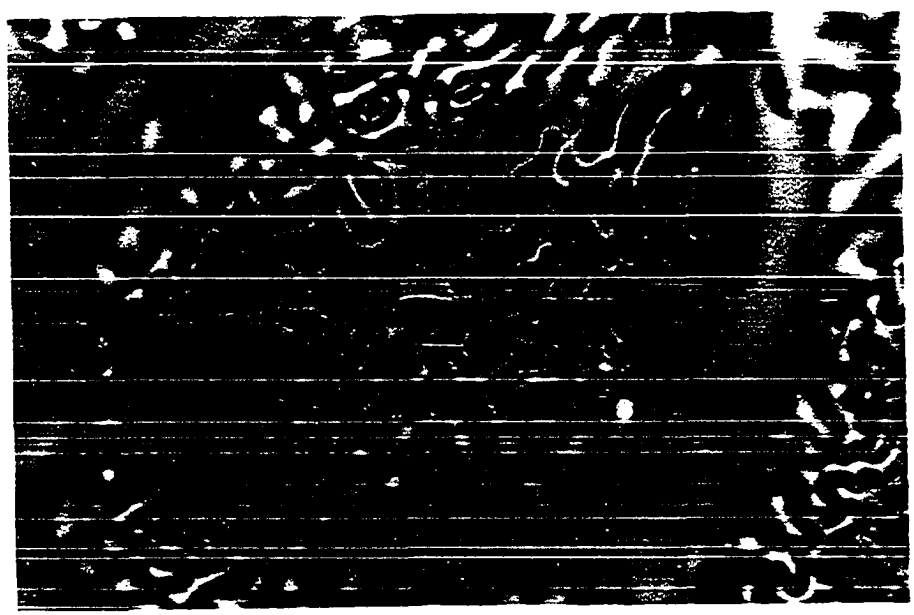
2. Density-thickness determinations

All leaves were examined for density-thickness (leaf dry weight/leaf area). In previous research (22), the entire leaf was dried at approximately 80°C and weighed. The density-thickness was expressed as g/dm^2. In 1969 and 1970, the entire leaf could not be used for density-thickness because part of the leaf lamina had to be used for anatomy measurements. Internal anatomy leaf sections were taken from the middle of the leaf lamina on each side of the midvein. This left the remainder

Figure 10. Photomicrograph of an impression of the adaxial leaf surface of an Amsoy leaf tested on August 13, 1970. Picture taken at 250x and enlarged to 268x. 1 cm = 37 μ

Figure 11. Photomicrograph of an impression of the abaxial leaf surface of an Amsoy leaf tested on August 13, 1970 (same leaf as in Figure 10). Picture taken at 250x and enlarged to 268x. 1 cm = 37 μ





of the leaf for density-thickness determinations. Four leaf punches were taken from each leaf with a no. 10 cork borer. These leaf punches were placed in capped vials and left at room temperature until the end of the testing period for the day. After leaf punches were dried for 24 hours at approximately 80°C, dry weights were taken.

Leaf thickness was determined on every leaf tested by a mechanical micrometer with a readibility of one ten-thousandth of an inch. Equal tension could be applied to every leaf because the micrometer had a tension rachet. Thickness was determined immediately after the silicone rubber was applied upon removal from the chamber. It was determined in three locations where no "major" veins were present.

3. Preparation of leaf cross-sections

Leaf cross-sections were prepared differently in 1969 and 1970. In 1969, free-hand cross-sections[1] were prepared, killed, fixed, and mounted. Free-hand sections were prepared by using a potato as a pith stick and slicing the potato and leaf simultaneously. By this method, it is possible to get sections only several cell layers thick (less than 50 μ).

The free-hand sections were placed immediately in 95% ethanol for killing and bleaching. Woven wire baskets were used for ease of transfer from one solution to the next. Ethanol fixes the tissue in a reasonably natural state. After five minutes in 95% ethanol, the sections were placed, briefly (5-10 sec), in a 1% Fast Green in 95% ethanol. Fast Green

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[1]Lersten, N. R., Ames, Iowa. Cross-section technique. Private communication. 1969.

stains the cells sufficiently for indentification. After staining, the sections were rinsed for 15-30 sec in 100% ethanol. Destaining followed in a 1:1 solution of xylene:100% ethanol (v/v) for one to two minutes. After destaining, the sections were moved to xylene for over three minutes to prevent stain from dissolving out and to prepare for mounting. Sections were mounted in a xylene-soluble resin, piccolyte. Slides were stored horizontally for later examination and measurements.

Leaf cross-sections in 1970 were prepared by free-hand sectioning without a pith stick. The thicker sections were desirable because they were cleared the same as leaf clearings explained in the next section.

Leaf cross-sections and clearings were photographed through the microscope at 400x with bright-field illumination and green filter. The photomicrographs were treated the same as for epidermal impressions mentioned earlier. In 1969, drawings were made from the projected image with a magnification of 1600x. In 1970, enlarged photographic prints were made at the same magnification. Figure 12 illustrates a cleared cross-section of an Amsoy leaf.

4. Preparation of leaf clearings

Leaf clearings were prepared for the paradermal view of the leaves. It was believed this would give a three-dimensional perspective of the internal anatomy. After focusing on certain layers within the leaf, paradermal photographs were taken. Soybean mesophyll consists of two palisade parenchyma layers, a paraveinal layer, and a spongy parenchyma region (33, 34). These different regions are illustrated by Figures 13, 14, 15, and 16.

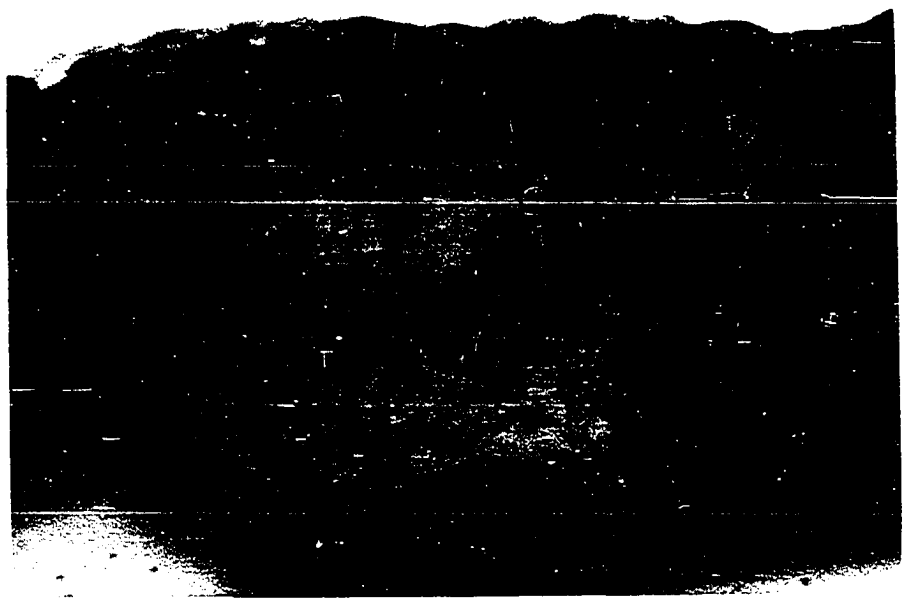
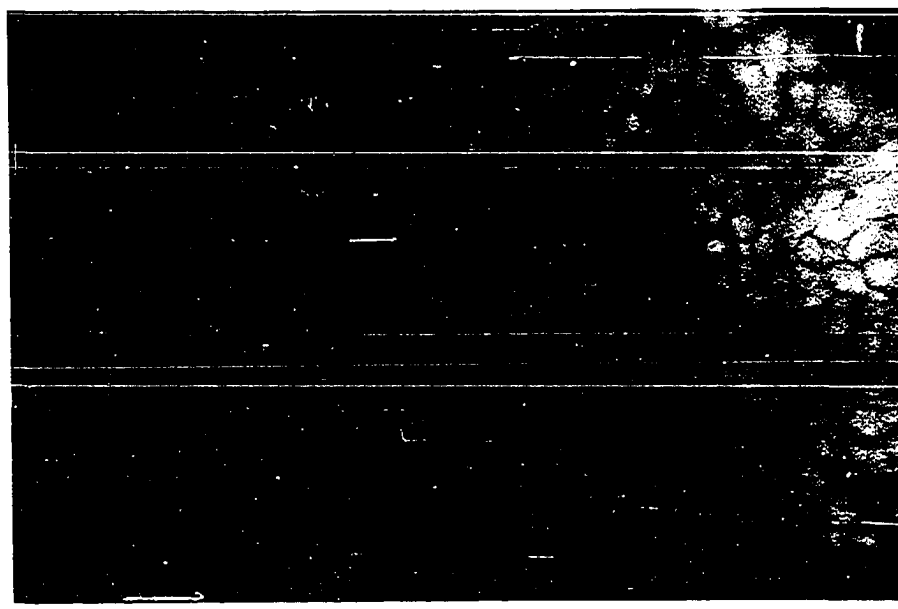


Figure 12. Photomicrograph of a leaf clearing in cross-sectional view. Amsoy leaf tested July 23, 1970. Picture taken at 400x and enlarged to 442x. 1 cm = 23 μ. (upper epidermis, A; upper palisade parenchyma, B; lower palisade parenchyma, C; paraveinal mesophyll, D; spongy parenchyma, E; and lower epidermis, F)

Figure 13. Photomicrograph of a leaf clearing from a paradermal perspective (looking down on the leaf). Amsoy leaf tested August 6, 1970 showing the upper palisade parenchyma cells. Picture taken at 400x and enlarged to 442x. 1 cm = 23 μ

Figure 14. Photomicrograph same as Figure 13 except focused on the lower palisade parenchyma cells within the Amsoy leaf



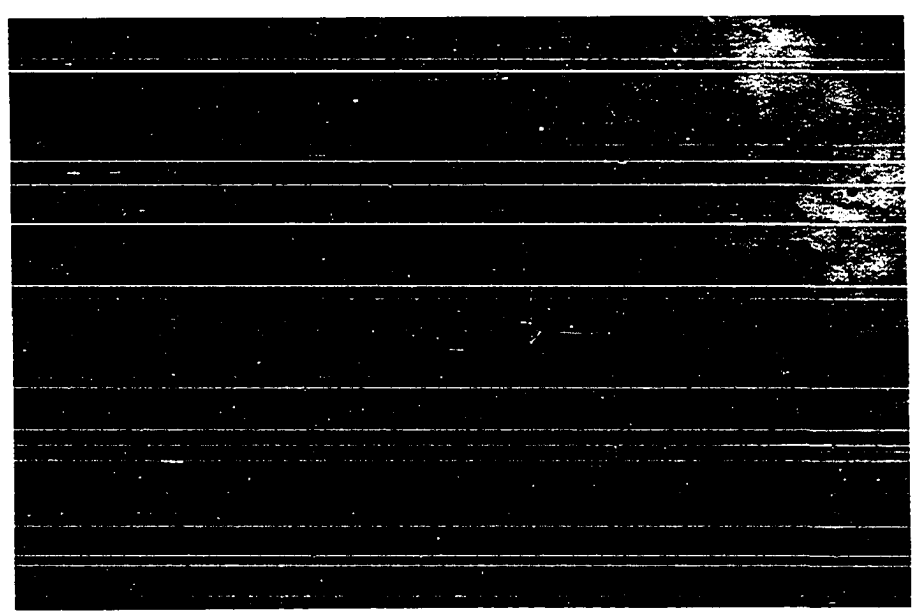
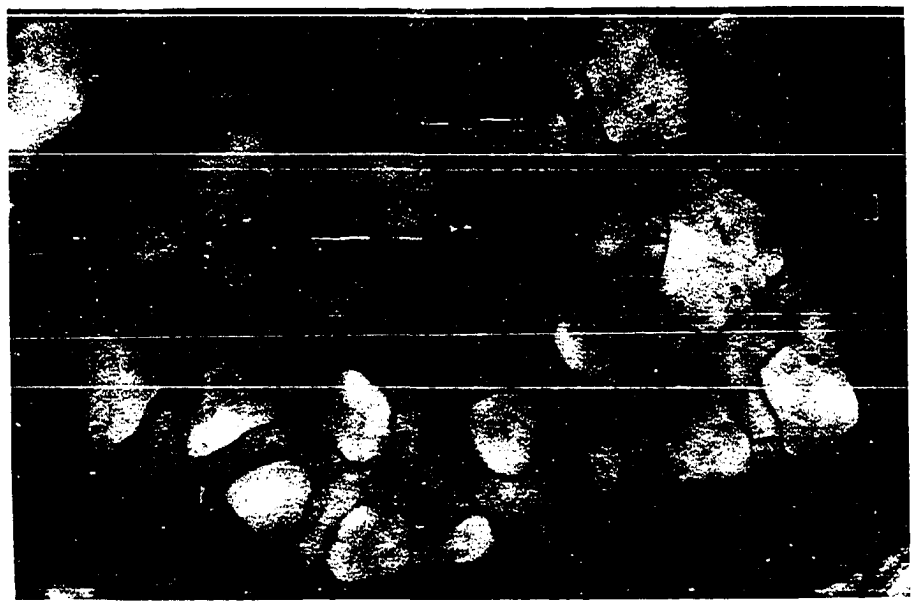
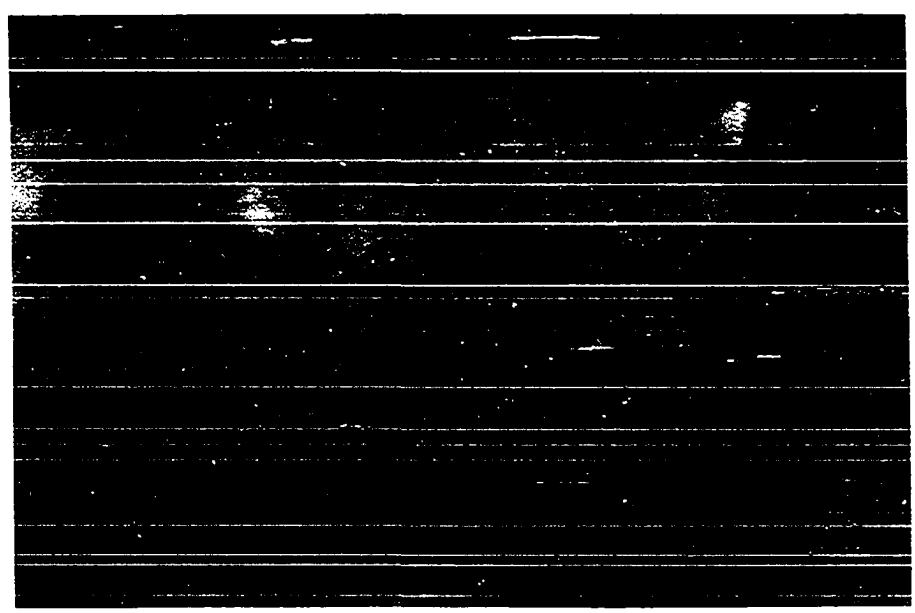


Figure 15. Photomicrograph of a leaf clearing from a paradermal view. Amsoy leaf tested July 30, 1970; illustrating the paraveinal mesophyll cells. Picture taken at 400x and enlarged to 442x. 1 cm = 23 μ

Figure 16. Photomicrograph of a leaf clearing from a paradermal view. Amsoy leaf tested July 23, 1970; illustrating the spongy parenchyma cells. Picture taken at 400x and enlarged to 442x. 1 cm = 23 μ





Leaf clearings[1] were prepared because they gave good resolution of cellular dimensions. About a square centimeter of leaf lamina was placed into 95% ethanol for about a day to remove the chlorophyll. The remainder of cell contents were removed by two days in 10% NaOH. In 1969, leaf sections also were bleached in chlorox for a minute. Both years, bleaching was followed by three five-minute rinses of distilled water. Sections then were placed in aqueous chlorol hydrate (250 g/100 ml H_2O) for a day or more. Sections were stored in this solution until it was convenient to finish the clearings. Leaf clearings were rinsed in three changes of distilled water, by a dilution series. Sections were dehydrated in three changes of 95% ethanol for five minutes each. This was followed by five minutes in 100% ethanol. Prior to staining, the sections were placed for five minutes in a 1:1 (v/v) solution of xylene:100% ethanol. After five minutes in a staining solution of 1% safranin in a 1:1 solution of xylene and 100% ethanol, the leaf clearings were destained in a 1:1 solution of xylene:100% ethanol. Sections were then placed in xylene for a few minutes to stop destaining. Some problems were encountered with safranin precipitation within the leaf clearings, and some sections had to be restained. Clearings were mounted in piccolyte for horizontal storage.

5. Measurement and calculations of internal anatomy

For stomatal measurements in 1969, the entire area on the photographs was used, or approximately 6.5×10^{-2} mm^2. From this sample area per

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[1]Lersten, N. R., Ames, Iowa. Leaf clearing procedure. Private communication. 1969.

leaf, 3 to 10 stomata were measured (width and length) and the entire number of stomata were counted per sample area. In 1970, about 3.2 x 10^{-2} mm^2 was used for a sample.

All other anatomical measurements were made on a leaf section of 1.21 x 10^4 μ^2. This section was assumed to represent the entire leaf. The leaf section was square (1.1 x 10^2 μ X 1.1 x 10^2 μ). Thus the cross-sectional area examined was also 1.1 x 10^2 μ in width. The anatomical measurements and calculations were similar to those used by Turrell (98, 99). Turrell's procedure was slightly modified to fit the purpose of the study. There was also some major modifications between 1969 and 1970. When the paraveinal region was discovered, the anatomical measurements and calculations were changed to accommodate this additional defined layer. 1969 calculations were not changed to include this additional layer of cells, because the leaf cross-sections were not clear enough (poor resolution) for this change.

Many measurements were taken on the defined sample of leaf tissue. Diameter of the palisade cells was taken on about ten cells per leaf. Cell length or thickness of the different cellular regions was measured as well. Length of upper palisade parenchyma cells was taken to represent the thickness of this region. Only cells of reasonable resolution were measured. Thickness of the lower palisade layer was measured in a similar manner. The paraveinal mesophyll layer was a single layer of cells in the plane of the phloem and xylem (see Figures 12 and 15). Thickness of this tissue was determined in three different places from the cross-sectional view in 1970 only. Thickness of the spongy mesophyll region (paraveinal

and spongy mesophyll combined in 1969) was determined also in the cross-sectional view in approximately three locations. Leaf thickness was also determined on the leaf clearings in three locations. A microscope stage micrometer was used to measure thickness by focusing on the adaxial surface and then on the abaxial surface.

Internal exposed surface areas of the various regions within the leaf were also of interest. In 1970, these parameters were estimated by measuring the total length of exposed cell walls (not bordering other cells) in the defined sample area per paradermal region. For each region the thickness was measured, as mentioned earlier, so a simple multiplication gives an estimate of exposed surface area (Equation 11, 12, 13, and 14). The paradermal photomicrographs (Figures 13, 14, 15, and 16) were made at approximately the middle of the cellular layers.

$$S_U = L_U \cdot p_U \cdot k \qquad (11)$$

S_U = exposed cell surface area of upper palisade parenchyma sample volume, μ^2

L_U = length of upper palisade parenchyma cells, μ

p_U = total length of exposed cell walls (not bordering cell walls) from a paradermal view of the defined sample volume (1.21×10^4 μ^2 x leaf thickness, T_c) of the upper palisade layer, cm

$k = 6.25 \frac{\mu}{cm}$, which is a conversion factor for magnification (1600x) and cm to μ meters

$$S_L = L_L \cdot p_L \cdot k \qquad (12)$$

S_L = exposed cell surface area of lower palisade parenchyma sample volume, μ^2

$$S_{PV} = L_{PV} \cdot p_{PV} \cdot k \qquad (13)$$

S_{PV} = exposed cell surface area of paraveinal mesophyll sample volume, μ^2

$$S_S = L_S \cdot p_S \cdot k \qquad (14)$$

S_S = exposed cell surface area of spongy parenchyma sample volume in 1970, μ^2

$$S_S = L\left[\left(\frac{h_t \cdot C \cdot 39.06}{h_n}\right) + \left(2.42 \cdot 10^4 - 10.08 \cdot A_S\right)\left(\frac{l_e}{l_t}\right)\right] \qquad (15)$$

S_S = exposed cell surface area of spongy parenchyma sample volume in 1969, μ^2

L = average number of spongy mesophyll cell layers (from cross-sectional view), integers

h_t = total length of exposed cell walls making an angle $<45^\circ$ with vertical for sample area (from cross-sectional view), cm

h_n = total number of vertically exposed cell walls for sample area (from cross-sectional view), integers

C = total length of spongy cell walls exposed for defined sample area (from the paradermal view), cm

A_S = total area of intercellular space for defined sample area (from the paradermal view), planimeter integers

l_e = total length of exposed spongy cell walls making an angle $>45^\circ$ with vertical for the defined sample area (from the cross-sectional view), cm

l_t = total length of spongy cell walls (exposed + unexposed) making an angle $>45^\circ$ with vertical for defined sample area (from the cross-sectional view), cm

39.06 = magnification factor and cm to μ conversion factor for h_t and C

2.42×10^4 = 2 x leaf sample area (1.21×10^4 μ^2)

10.08 = magnification factor and constant to convert planimeter reading of A_S to μ^2

In 1969, the exposed surface area of the spongy parenchyma layer was calculated by a more elaborate method, similar to that of Turrell (98). In 1970, the method was simplified because the simplified technique was easier and was believed sufficiently accurate for the purposes of the study. Equation 15 was used for the calculation of spongy parenchyma surface area. 1969 anatomical measurements of the spongy parenchyma layer are in error because the paradermal section used for the measurements was actually the paraveinal cell layer[1]. Originally, it was thought that this was the spongy parenchyma. Hence, the 1969 measurement of spongy mesophyll is an estimate of the internal exposed surface area of the paraveinal and spongy regions combined. In 1969, some horizontally oriented cells were observed, but as it is a highly disorganized region, they were thought merely part of the spongy parenchyma.

Volume of intercellular space is also of interest in this research. Volume of intercellular space per sample volume was estimated by measuring the area occupied by air in the paradermal sections and, then, multiplying by the thickness of the specific layer. For the palisade and spongy layers, it was more convenient to measure the cellular area from the

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[1]The author is indebted to Jerry G. Criswell for his bringing to my attention the Ph.D. thesis by Fisher (33).

paradermal sections, and volume of intercellular space was determined by subtracting cellular volume per sample volume from the sample volume (Equation 16, 17, and 18). It is more efficient to measure the area occupied by air space in the paradermal section of the paraveinal layer (Equation 19).

$$V_U = L_U(1.21 \cdot 10^4 - 5.039 \cdot A_U) \quad (16)$$

V_U = volume of intercellular space in upper palisade parenchyma layer per sample volume, μ^3

L_U = length of upper palisade layer, μ

A_U = total area of upper palisade parenchyma cells for the defined sample area (from the paradermal view), integers

1.21×10^4 = sample area, μ^2

5.039 = magnification factor and constant to convert planimeter integers for A_U to μ^2

$$V_L = L_L(1.21 \cdot 10^4 - 5.039 \cdot A_L) \quad (17)$$

V_L = volume of intercellular space in lower palisade parenchyma layer, μ^3

$$V_S = L_S(1.21 \cdot 10^4 - 5.039 \cdot A_S) \quad (18)$$

V_S = volume of intercellular space in the spongy parenchyma layer, μ^3

$$V_{PV} = L_{PV} \cdot a_{PV} \cdot 5.039 \quad (19)$$

V_{PV} = volume of intercellular space in the paraveinal mesophyll layer, μ^3

a_{PV} = total area of intercellular space of the paraveinal mesophyll layer for a defined sample area (from the paradermal view), integers

Cellular volume is an important parameter, but it can be obtained by subtraction of volume of intercellular space from total volume of the tissue layer. Other interesting variables, e.g., surface/volume ratios, are obtained by division.

6. Error analysis

There are many possible sources of error in the anatomical measurements, but there is considerable variability present in the leaves as well. Probably the smallest error is instrument error. The planimeter used for measurement of areas in this research was a Filotecnica salmorragi Type 236. Absolute error is small with a standard circle, usually less than 0.4%. The chartometer used to follow the length of curved lines was a Tacro No. 4714. Error is small when checking it with a straight line--approximately 1%.

The larger error involves the ability to follow the lines with the instruments. This type of error was not measured or estimated. Probably another larger error is the occasional poor resolution of some cell walls. This is a problem, because missing observations are very critical when only four replications per variety are present. When part of the cell wall was obvious, the rest of it could be drawn with good assurance. The layer that has the poorest resolution, and consequently the most error, is the spongy mesophyll layer (Figure 16), so inferences drawn from these measurements will be limited.

Another error is assumption of negligible surface area of the ends of palisade cells. There is no way of knowing the magnitude of this error,

because many of the cells appear to abut others; however, this may be an artifact of microscopic observation. Assuming the middle of the various tissue layers represents the entire layer contributes another error. Also, exposed surface area of other cells in the leaf--e.g., epidermal cells--may contribute to the exposed surface area related to net photosynthesis, too. Epidermal cell area was not estimated.

The effect of killing, fixing, dehydrating, staining, and mounting on internal dimensions is unknown also. Using only one sample per leaf tested is a limitation, as is the small population of leaves tested for anatomy. Inspite of these limitations, it is believed the anatomical measurements should give some insight into net photosynthetic relationship to cellular anatomy.

IV. RESULTS

A. Statistical Analysis

Varietal differences in the numerous measured and calculated variables were determined by statistical analysis of variance. A completely randomized block design was used all three years. Blocks were treated as replications. One replication per day was performed in 1969 and 1970.

To facilitate varietal comparisons, a standard error of difference between treatment means was calculated, $s_{\bar{d}}$. This parameter allows one to use a suitable statistical test for treatment (variety) comparisons, such as an LSD test ($LSD = t \cdot s_{\bar{d}}$). To estimate the variability of a variable, the coefficient of variability, CV, was also calculated. These various statistical parameters are given in Table 32 of the Appendix.

The correlation of various variables was also of interest in this study. The linear relationship between two variables can be estimated by simple correlation coefficients, r. Simple correlation coefficients were obtained by generating correlation matrices of all experimental units within varieties, among varieties, and among varieties and years. To help assign a certain degree of cause and effect to the association of variables, partial correlation coefficients and multiple regression analysis were performed.

B. Net Photosynthesis

As mentioned in the introduction, part of the purpose of the research was to examine measured and calculated variables among years and varieties. Seasonal trends were examined also.

1. Variation among years and varieties

Since an estimate of variation due to years and varieties is desired, one must consider all other variables that changed among years and which may affect the results. Table 2 shows the average conditions under which leaves were tested each year. (The reader is referred to Table 1 for the definition of symbols and their units.) The leaves were probably under more stress (atmospheric demand) in 1969 than in other years. Higher leaf temperatures and higher vapor pressure deficits may have caused lower water potential in the leaves and, perhaps, some stomatal closure. Higher than optimum leaf temperature would result in alteration of the general metabolism of leaves, too. It is not known for certain whether these higher stress conditions did, in fact, significantly decrease net photosynthesis. Tests (see sections on response of leaves to humidity and temperature) indicated that these leaves were under near optimum conditions, but the tests were limited in replication and were not conducted under steady state conditions.

The most ideal conditions seem to be those of 1970 where leaf temperature equaled air temperature, and there was a low vapor pressure deficit between leaf and ambient air, and high windspeed. However, the light flux density was less in 1970 as a result of $CuSO_4$ addition to the infrared absorbing baths. A test with one leaf indicated that the addition of $CuSO_4$ to the baths had no immediately detectable effect on net photosynthesis. The test with and without $CuSO_4$ was performed with a greenhouse grown Harosoy leaf, with a net photosynthetic rate of 30, on April 5, 1970. It is possible that field grown plants of higher photosynthetic

Table 1. List of symbols, their definitions and units

Term or Symbol	Definition	Units
Light	Light flux density	$ergs \cdot cm^{-2} \cdot sec^{-1}$
Wind	Windspeed	$cm \cdot sec^{-1}$
$\Delta[CO_2]$-1	CO_2 differential at 0 ppm ambient CO_2	$ppm(v/v)CO_2$
$\Delta[CO_2]$-2	CO_2 differential at 320 ppm ambient CO_2	$ppm(v/v)CO_2$
Flow	Air flow rate into leaf chamber	$l \cdot hr^{-1}$
VPDA	H_2O vapor pressure deficit of air	mm Hg
VPDL	H_2O vapor pressure deficit between leaf and air	mm Hg
RH-IN	Relative humidity of chamber ambient air	%
RH-OUT	Relative humidity of chamber egress air	%
LT	Leaf temperature	°C
AIR T	Air temperature in leaf chamber	°C
P_{320}	Net photosynthesis at 320 ppm CO_2 on leaf area basis (one surface)	mg CO_2 dm^{-2} hr^{-1}
P_{wt}	Net photosynthesis at 320 ppm CO_2 on leaf dry weight basis	mg CO_2 g^{-1} hr^{-1}
DAY	Days after June 30	Days
R_o	CO_2 evolution into 0 ppm CO_2 air in light	mg CO_2 dm^{-2} hr^{-1}
R_c	Estimate of photorespiration	mg CO_2 dm^{-2} hr^{-1}
Γ	CO_2 compensation concentration	ppm CO_2
P_i	Estimate of "true" photosynthesis	mg CO_2 dm^{-2} hr^{-1}
Tr	Transpiration rate	g H_2O dm^{-2} hr^{-1}
P/Tr	Net photosynthesis at 320 ppm/transpiration	$x\ 10^{-2}$g $CO_2 \cdot$g H_2O^{-1}

Table 1. (Continued)

Term or Symbol	Definition	Units
$[CO_2]_{chl}$	Concentration of CO_2 at the site of fixation	ppm CO_2
$\sum r_o$	Sum of resistance to CO_2 diffusion with $[CO_2]_{chl}$ = 0 ppm CO_2	$sec \cdot cm^{-1}$
$\sum r_\Gamma$	Sum of resistance to CO_2 diffusion with $[CO_2]_{chl} = \Gamma$	$sec \cdot cm^{-1}$
$r_a + r_s$	Laminar and stomatal resistance to CO_2 diffusion	$sec \cdot cm^{-1}$
r_a	Laminar resistance to CO_2 diffusion	$sec \cdot cm^{-1}$
r_s	Stomatal resistance to CO_2 diffusion	$sec \cdot cm^{-1}$
r_{mo}	Mesophyll resistance to CO_2 diffusion, $[CO_2]_{chl}$ = 0 ppm	$sec \cdot cm^{-1}$
$r_{m\Gamma}$	Mesophyll resistance to CO_2 diffusion, $[CO_2]_{chl} = \Gamma$	$sec \cdot cm^{-1}$
S	Slope of net photosynthesis-CO_2 curve	$\frac{mg\ CO_2}{dm^2 \cdot hr \cdot ppm\ CO_2}$
DT	Leaf density-thickness	$g \cdot dm^{-2}$
DT-V	Leaf density-thickness over midvein	$g \cdot dm^{-2}$
TH	Leaf thickness in inches	$\times 10^{-4}$ in
Area	Area of terminal leaflets	dm^2
W_{ad}	Width of stomata on adaxial surface	μ
W_{ab}	Width of stomata on abaxial surface	μ
L_{ad}	Length of stomata on adaxial surface	μ
L_{ab}	Length of stomata on abaxial surface	μ
N_{ad}	Density of stomata on adaxial surface	no. of stomata/mm^2

Table 1. (Continued)

Term or Symbol	Definition	Units
N_{ab}	Density of stomata on abaxial surface	no. of stomata/mm^2
T_m	TH in microns	μ
T_c	Thickness of lamina by microscope micrometer from leaf clearings	μ
L_U	Length of upper palisade parenchyma cells	μ
L_L	Length of lower palisade parenchyma cells	μ
L_{PV}	Thickness of paraveinal mesophyll layer	μ
L_S	Thickness of spongy parenchyma layer	μ
D_U	Diameter of upper palisade parenchyma cells	μ
D_L	Diameter of lower palisade parenchyma cells	μ
S	Exposed cell surface area of sample volume	x 10^4 μ^2
S_U	S for upper palisade parenchyma layer	x 10^4 μ^2
S_L	S for lower palisade parenchyma layer	x 10^4 μ^2
S_{PV}	S for paraveinal mesophyll layer	x 10^4 μ^2
S_S	S for spongy parenchyma layer	x 10^4 μ^2
S_T	Total exposed surface area for sample volume	x 10^4 μ^2
S_E	External surface area of sample volume	x 10^4 μ^2
V	Volume of intercellular space in sample volume	x 10^4 μ^3
V_U	V for upper palisade parenchyma layer	x 10^4 μ^3
V_L	V for lower palisade parenchyma layer	x 10^4 μ^3

Table 1. (Continued)

Term or Symbol	Definition	Units
V_{PV}	V for paraveinal mesophyll layer	x 10^4 μ^3
V_S	V for spongy parenchyma layer	x 10^4 μ^3
V_T	Total volume of intercellular space in sample volume	x 10^4 μ^3
V_E	Volume of sample	x 10^4 μ^3
V_{EU}	V_E for upper palisade parenchyma layer	x 10^4 μ^3
V_{EL}	V_E for lower palisade parenchyma layer	x 10^4 μ^3
V_{EPV}	V_E for paraveinal mesophyll layer	x 10^4 μ^3
V_{ES}	V_E for spongy parenchyma layer	x 10^4 μ^3
V_{ET}	Total sample volume	x 10^4 μ^3
V_C	Cellular volume in sample volume	x 10^4 μ^3
V_{CU}	V_C for upper palisade parenchyma layer	x 10^4 μ^3
V_{CL}	V_C for lower palisade parenchyma layer	x 10^4 μ^3
V_{CPV}	V_C for paraveinal mesophyll layer	x 10^4 μ^3
V_{CS}	V_C for spongy parenchyma layer	x 10^4 μ^3
V_{CT}	Total cellular volume for sample	x 10^4 μ^3

capacity may have responded differently to the test. To test the 1970 chamber relative to the 1969 chamber, the same Harosoy leaf's net photosynthetic rate, with $CuSO_4$ in the water bath, was measured and the leaf was then inserted into the 1969 chamber and net photosynthesis measured again. The approximate difference in net photosynthesis was 1.7 mg CO_2 dm^{-2} hr^{-1}, with the 1970 chamber giving the highest rate.

Table 2. Means over varieties and replications of several variables for three years. See Appendix for statistics. Each mean (except Light and Wind) represents 300, 96, and 156 observations for 1968, 1969, and 1970. Terms and symbols are defined in Table 1

Year	Light	Wind[a]	$\Delta[CO_2]$-1	$\Delta[CO_2]$-2	Flow	VPDA	VPDL	RH-IN	RH-OUT	LT	AIR T
1968	34[b]	3[b]	—	33[b]	434[b]	12.4[bc]	17.5[bc]	40[bc]	60[bc]	29[+]	26[b]
1969	34[b]	16[b]	7[b]	27[b]	508[b]	16.5	21.3	27	46**	32	29
1970	26[b]	138[b]	8[b]	40[b]	442[b]	7.0**	7.0**	47	73**	26*	26
$\bar{x}$	31.3	52.3	7.5	33.3	461	12.0	15.3	38	60	29	27

[a]Mean windspeed by cross-sectional area of chamber, 1968; anemometer in 1969 and 1970.

[b]Not analyzed statistically.

[c]Means of chamber 2 only.

[+]Significant varietal variation ($P<0.10$).

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

The experimental evidence given here does not necessarily imply that all of the yearly variation in net photosynthesis is a result of experimental technique. The results could be confounded because an effort was made to improve the experimental conditions. In the literature review, it was stressed that there is a multitude of factors which may affect photosynthesis either directly or indirectly. Many of these factors of control may be modified by environment, and the environment is obviously different from year to year.

Yearly variation in net photosynthesis probably is primarily a combination of effect of environment during growth and testing. Experimental evidence suggests that much of the variation is a result of environment during growth. Table 3 presents the mean photosynthetic rates for the three years on a leaf area and dry weight basis. For yearly comparisons, the 1968 photosynthetic means (22, 23) have been adjusted from 300 to 320 ppm CO_2. In 1968 and 1970, varietal differences on a dry weight basis occurred also.

Since four varieties were common to each year of testing, their photosynthetic rates are compared in Table 4 and Figure 17. Photosynthetic rates of two other varieties, tested in 1970, also are given in Table 4. Net photosynthesis on a leaf area basis seems quite variable among years. Evidently, there is a variety by year interaction, or more likely, a variety by enviroment interaction. High photosynthesizing varieties were more variable than the low photosynthesizing varieties.

Table 3 indicates that P_{wt} was lowest in 1968. This might be explained partially by the fact that entire leaves were used for dry

Table 3. Mean yearly net photosynthetic rates on leaf area basis (P_{320}) and dry weight basis (P_{wt}). Experimental conditions are given in Table 2

Year	P_{320}	P_{wt}[a]	No. of Varieties	No. of Reps.
1968[b]	38**	95*	20	15
1969	36	120	4	24
1970	40**	153*	6	26
$\bar{x}$	38	123		

[a]Dry weight of entire leaf in 1968 and from leaf punches in 1969 and 1970.

[b]Adjusted to 320 ppm CO_2, analysis of variance performed for 300 ppm CO_2.

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

weight measurements in 1968, but leaf punches were used in 1969 and 1970. Leaf punches do not include the larger veins in their weight. This, however, does not explain the difference between 1969 and 1970 results.

2. Seasonal trends

Net photosynthesis also seems to vary within a year. Figures 18 to 21 show seasonal trends in net photosynthesis for four varieties tested three years. Table 5 presents simple correlation coefficients of net photosynthesis with days after June 30 and the same variable squared ($days^2$). These coefficients indicate that, in general, net photosynthesis increases during the season. These trends, however, were quite variable--e.g., 1968 correlations were generally higher than in 1969 and 1970. The

Table 4. Mean varietal net photosynthetic rates on leaf area, P_{320}, and leaf dry weight, P_{wt}, bases. Experimental conditions were as listed in Table 2. Varieties were significantly different in 1968 and 1970 and not in 1969

Variety	Year	P_{320}	P_{wt}
Corsoy	1968	47	102
	1969	36	119
	1970	44	155
	$\bar{x}$	42	125
Amsoy	1968	45	100
	1969	38	119
	1970	40	149
	$\bar{x}$	41	123
Hawkeye	1968	34	89
	1969	36	124
	1970	37	147
	$\bar{x}$	36	120
Richland	1968	35	100
	1969	34	118
	1970	34	147
	$\bar{x}$	34	122
Provar	1970	42	156
Lindarin	1970	42	165

developmental stage of the plant and the age of the leaves seemed to sometimes significantly affect the photosynthetic rate. Experimental data (Experimental Procedure section) indicated that the youngest, fully-expanded leaves were tested until Aug. 14-18 (Day 45-49) when leaves began to age (days after full expansion). Figures 18 to 21 indicate that net photosynthesis began to decrease around Day 50, 1969. This decline in

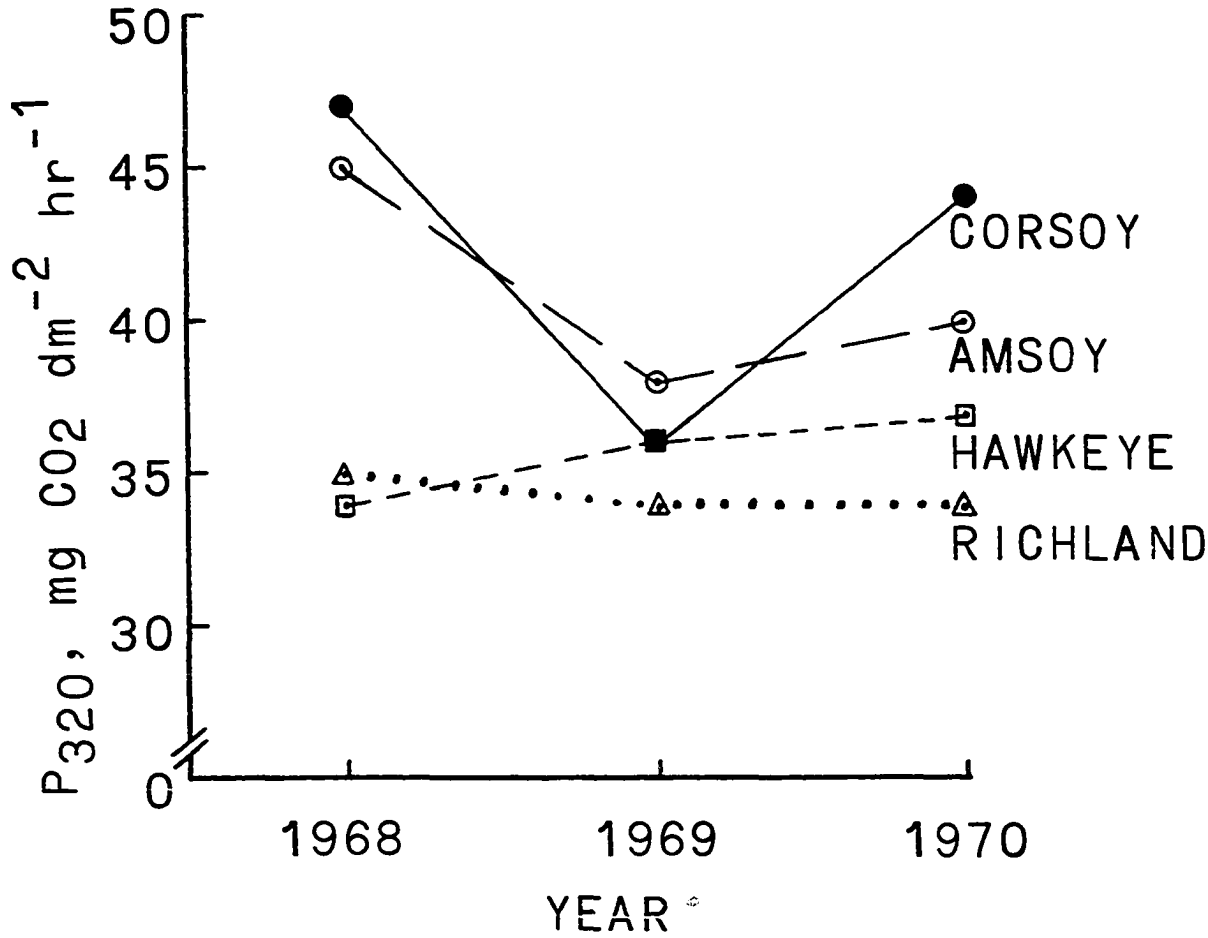

Figure 17. Yearly variation in mean varietal net photosynthetic rates, P_{320}. Experimental conditions are given in Table 2

Figure 18. Variation in net photosynthesis of Corsoy during the seasons of 1968, 1969, and 1970. Experimental conditions were as given in Table 2. Each point represents one measurement (leaf). Arrow indicates date of beginning seed formation stage (largest pod full length with seeds beginning to develop)

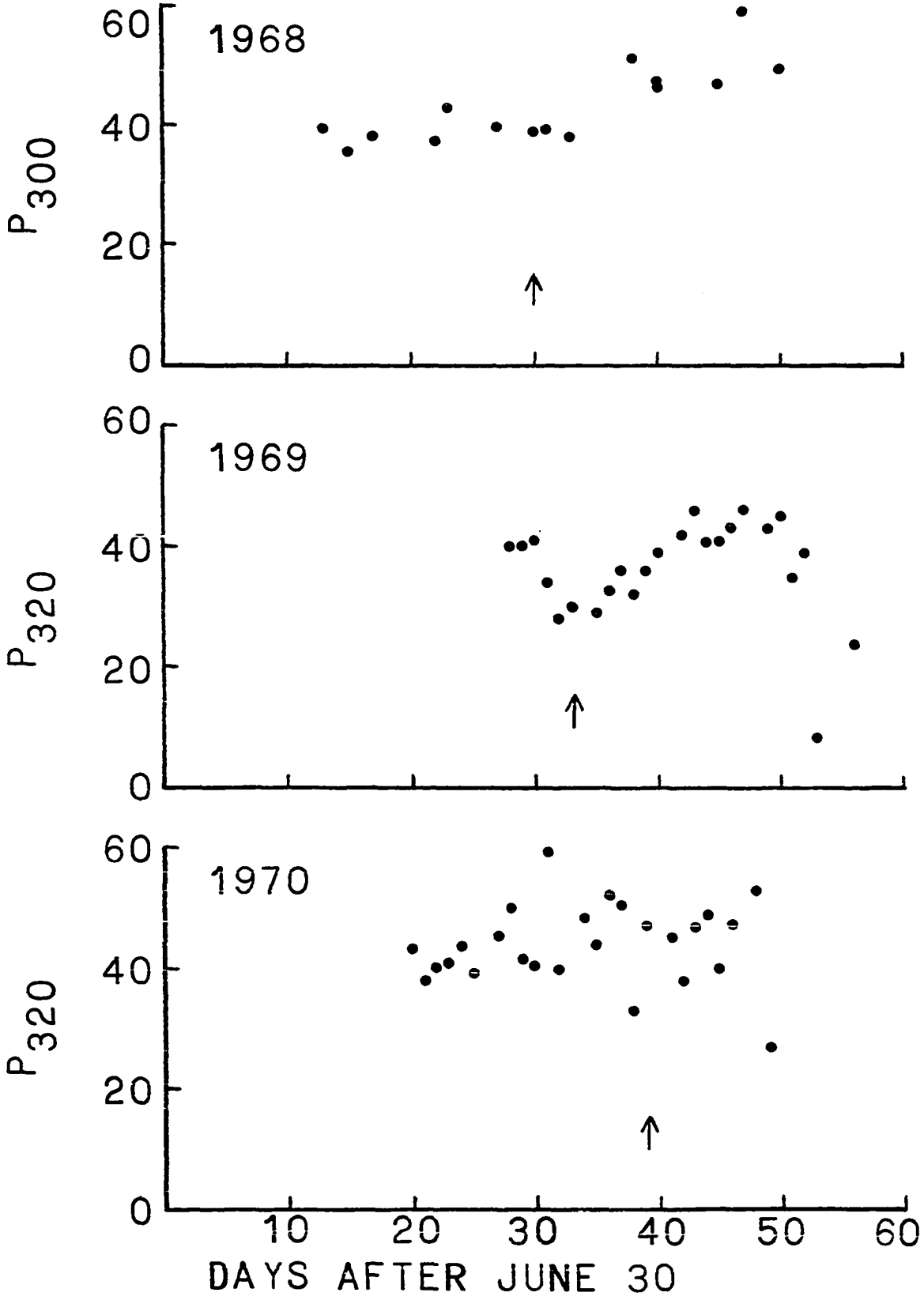
1968
1969
1970
P_{300}
P_{320}
P_{320}
0
20
40
60
10
20
30
40
50
60
DAYS AFTER JUNE 30

Figure 19. Variation in net photosynthesis of Amsoy during the seasons of 1968, 1969, and 1970. Experimental conconditions were as given in Table 2. Each point represents one measurement (leaf). Arrow indicates date of beginning seed formation stage (largest pod full length with seeds beginning to develop)

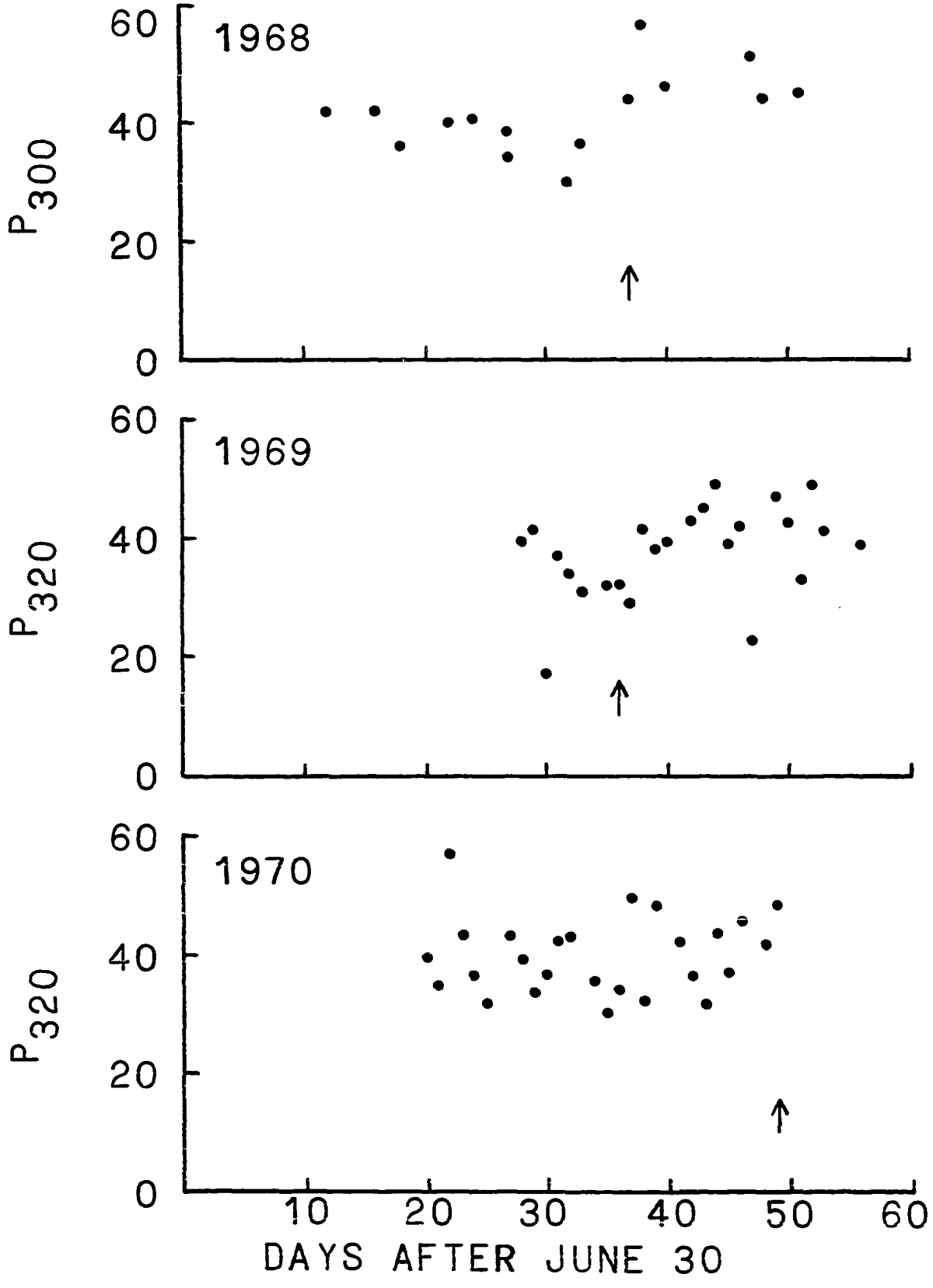
1968
1969
1970
P_{300}
P_{320}
P_{320}
0
20
40
60
10
20
30
40
50
60
DAYS AFTER JUNE 30

Figure 20. Variation in net photosynthesis of Hawkeye during the seasons of 1968, 1969, and 1970. Experimental conditions were as given in Table 2. Each point represents one measurement (leaf). Arrow indicates date of beginning seed formation stage (largest pod full length with seeds beginning to develop)

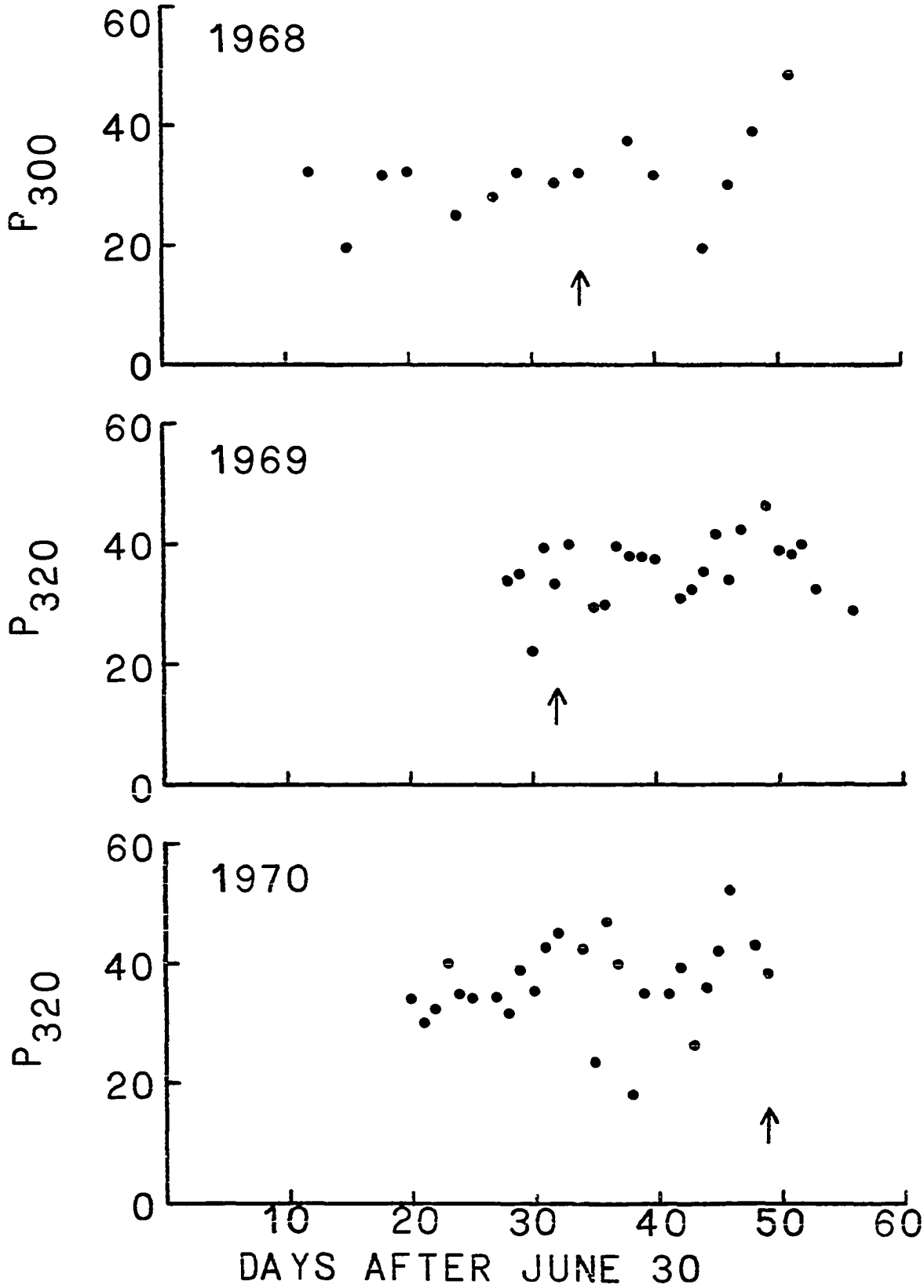
1968
1969
1970
P_{300}
P_{320}
P_{320}
60
40
20
0
10
20
30
40
50
60
DAYS AFTER JUNE 30

Figure 21. Variation in net photosynthesis of Richland during the seasons in 1968, 1969, and 1970. Experimental conditions were as given in Table 2. Each point represents one measurement (leaf). Arrow indicates date of beginning seed formation stage (largest pod full length with seeds beginning to develop)

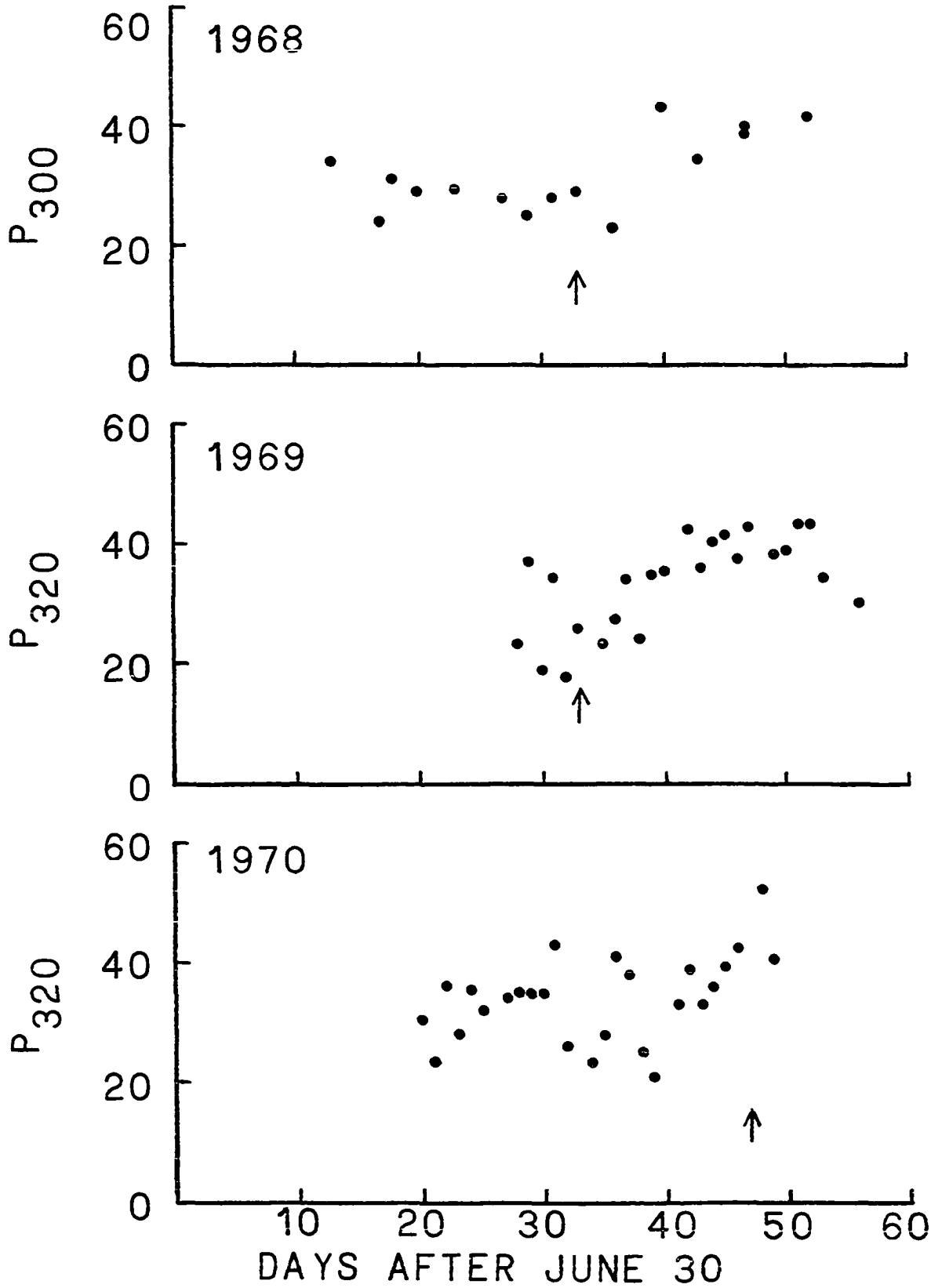
1968
1969
1970
P_{300}
P_{320}
P_{320}
60
40
20
0
10
20
30
40
50
60
DAYS AFTER JUNE 30

Table 5. Simple correlation coefficients between net photosynthesis (P_{300} and P_{320}) and days after June 30 (Day) and $(Day)^2$. Correlations are between individual observations within and among varieties

Variety	Year	Day	Day^2
Corsoy	1968	.80**	.83**
	1969	-.10	-.13
	1970	.05	.01
Amsoy	1968	.46	.50
	1969	.39	.38
	1970	.08	.11
Hawkeye	1968	.45	.48
	1969	.27	.24
	1970	.24	.25
Richland	1968	.62*	.69**
	1969	.62**	.60**
	1970	.43*	.46*
Provar	1970	.31	.32
Lindarin	1970	.46*	.47*
Among Varieties	1968	.45**	.48**
	1969	.28**	.26*
	1970	.22**	.23**

*Significant ($P<0.05$).

**Significant ($P<0.01$).

net photosynthesis may be a result of declining single leaf efficiency (leaf age) or the plant's physiological stage of development (plant age).

Insertion level of the leaves on the main stem seems to affect significantly the rate of net photosynthesis. The leaf insertion level effect could be related to physiological stage of development of the plant. The seasonal trends of 1968 may be related to the increased

demand for photosynthate by pods. This is suggested because the sudden increase in net photosynthesis that occurs around the first week of August coincides with the beginning of pod-filling (bean growth).

Seasonal trends in 1969 are more complicated. Net photosynthesis, in general, increases around the first week in August, as in 1968, but it seems merely recovering from an earlier depression in photosynthesis. That is, initial measurements of photosynthesis were high, too. There were no significant time trends in 1970.

C. Light Respiration

The two estimates of light respiration used in this research, R_o (CO_2 evolution in zero-CO_2 and light) and R_c (R_o adjusted for CO_2 diffusion resistances), are considered minimal estimates. Tables 6 and 7 show the variation in estimates of light respiration. CO_2 evolution, R_o, was much lower in 1968 compared to 1969 and 1970. It is possible that this difference may be a result of lower windspeed in 1968, but if so, photosynthesis should have been lower too. Varietal differences in R_o were evident all three years. Also R_o was correlated with P_{320} in 1969 and 1970 (Table 8). CO_2 evolution increased during the season.

Adjusted CO_2 evolution, R_c, is considered a better estimate of photorespiration (89). Its seasonal variation was very large, compared to R_o, in 1968 but not in 1970 (see Appendix, Table 32). In general, R_c varied similarly to R_o, statistically, except it was larger in magnitude. R_c was much higher in 1969 than in 1968 and 1970. True photosynthetic rate, P_i, was estimated by addition of P_{320} and R_c.

Table 6. Mean yearly estimates of light respiration. Experimental conditions were as given in Table 2. (CO_2 evolution in zero-CO_2 and light, R_o; R_o adjusted for diffusion resistances, R_c; true photosynthesis, P_i; CO_2 compensation concentration, Γ)

Year	R_o	R_c	P_i	R_c/P_i	Γ
1968[a]	5.6+	13	51**	.26	40
1969[a]	9.0+	20[b]	56[b]	.36[b]	65
1970	8.3**	11**	50**	.22[b]	56*
$\bar{x}$	7.6	15	52	.29	54

[a]1968 and 1969 variables R_c, P_i, and R_c/P_i are calculated from yearly means. 1968 are for chamber 2 only.

[b]Not analyzed statistically.

+Significant varietal variation ($P<0.10$).

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

The proportion of true photosynthesis accounted for by respiration R_c/P_i was approximately 29%. In 1969, a higher proportion of photosynthate was photorespired than the other years. There were no varietal differences in R_c/P_i in 1968, the one year this parameter was analyzed statistically.

The magnitude of the CO_2 compensation concentration, Γ, also, may indicate the magnitude of photorespiration. Γ was lowest in 1968 and highest in 1969. Varietal differences in Γ were demonstrated only in 1970. Γ seems negatively correlated to net photosynthesis and positively correlated to R_o (Table 8).

In summary, net photosynthesis seems positively related to R_o and R_c and negatively correlated to Γ.

Table 7. Mean varietal estimates of light respiration, true photosynthesis, and proportion of true photosynthesis accounted for by R_c (diffusion resistance adjusted CO_2 evolution). Significance of varietal variation given in Table 6. Experimental conditions as given in Table 2

Variety	Year	R_o	R_c	Γ	P_i	R_c/P_i
Corsoy	1968	6.4	16	37	63	.25
	1969	9.1	21	66	57	.37
	1970	8.9	12	54	56	.21
	$\bar{x}$	8.1	16	52	59	.28
Amsoy	1968	5.8	15	37	60	.25
	1969	9.7	23	67	61	.38
	1970	8.3	11	56	51	.22
	$\bar{x}$	7.9	16	53	57	.28
Hawkeye	1968	5.5	13	41	47	.28
	1969	8.8	19	63	55	.35
	1970	8.1	10	58	47	.21
	$\bar{x}$	7.5	14	54	50	.28
Richland	1968	5.0	11	39	46	.24
	1969	8.4	19	66	53	.36
	1970	7.7	10	60	44	.23
	$\bar{x}$	7.0	13	55	48	.28
Provar	1970	8.5	11	54	53	.21
Lindarin	1970	8.4	11	54	52	.21

D. Diffusion Resistances

Diffusion resistances were calculated for all three years (Tables 9 and 10). Correlation coefficients of several resistance related variables are given in Table 11. Transpiration rates are presented in this section, because they were required for the calculation of diffusion resistances. Net photosynthesis-transpiration ratios were calculated as well.

Table 8. Simple correlation coefficients with estimates of light respiration between individual observations within and among varieties. (CO_2 evolution in zero-CO_2 and light, R_o; days after June 30, Day; net photosynthesis, P_{320}; R_o adjusted for CO_2 diffusion resistances, R_c; CO_2 compensation concentration, Γ)

Variety	Year	R_o-Day	R_o-P_{320}	R_c-P_{320}	Γ-P_{320}	Γ-R_o	Γ-Day
Corsoy	1968	.50	—	—	-.13	.74**	-.13
	1969	.06	.55**	—	-.67**	-.16	.28
	1970	.39*	.20	.09	-.64**	.60**	.31
Amsoy	1968	.27	—	—	-.49	.35	-.49
	1969	.40*	.55**	—	-.60**	.33	-.03
	1970	.53**	.39*	.18	-.65**	.43*	.34
Hawkeye	1968	.58*	—	—	.31	.91**	.31
	1969	.26	.62**	—	-.24	.61**	.08
	1970	.02	.63**	.35	-.63**	.17	-.19
Richland	1968	.33	—	—	-.46	.78**	-.46
	1969	.07	.50*	—	-.74**	.20	-.67**
	1970	.38	.66**	.52**	-.63**	.16	-.16
Provar	1970	.44*	.62**	.63**	-.46*	.39*	.17
Lindarin	1970	.28	.45*	.44*	-.25	.74**	-.05
Among Varieties	1968	.48**	—	—	-.18**	.68**	-.18*
	1969	.19	.56**	—	-.59**	.26*	-.06
	1970	.32**	.55**	.42**	-.61**	.31**	.07

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 9. Mean yearly transpiration rate (Tr), net photosynthesis to transpiration ratios (P/Tr), slope of the CO_2 response curve (S), sum of resistances to diffusion of CO_2 assuming $[CO_2]_{chl} = 0$ ($\sum r_o$), sum of resistances to diffusion of CO_2 assuming $[CO_2]_{chl} = \Gamma$ ($\sum r_\Gamma$), sum of stomatal and laminar resistance ($r_a + r_s$), laminar resistances (r_a), stomatal resistances (r_s), mesophyll resistance assuming $[CO_2]_{chl} = 0$ (r_{mo}), and mesophyll resistance assuming $[CO_2]_{chl} = \Gamma$ ($r_{m\Gamma}$). Experimental conditions as given in Table 2

Year	Tr	P/Tr	S	$\sum r_o$	$\sum r_\Gamma$	$r_a + r_s$	r_a	r_s	r_{mo}	$r_{m\Gamma}$
1968[a]	3.3*	1.2[b]	.14**	6.0[b]	5.5**	3.1**	1.1[b]	2.0**	2.9[b]	2.4*
1969	4.0	0.9	.14+	6.8	5.3	3.0	0.9	2.1	3.8	2.4
1970	3.7**	1.1*	.15**	5.9**	4.9**	1.1**	0.9**	0.2[bc]	4.8[b]	3.8**
$\bar{x}$	3.7	1.1	.14	6.2	5.2	2.4	0.97	1.4	3.8	2.9

[a]1968 data was calculated from yearly means of chamber 2 only for variables Tr, P/Tr, $r_a + r_s$, r_a, r_s, r_{mo}, $r_{m\Gamma}$.

[b]Not analyzed statistically.

[c]Calculated from means.

+Significant varietal variation ($P<0.10$).

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

Transpiration rate, Tr, was correlated with photosynthetic rate. Loss of water from a leaf is a function of vapor pressure gradient from the evaporative surface within the leaf to the external edge of the boundary layer (VPDL). Transpiration is also affected by physical resistances to H_2O diffusion. Table 2 indicates that there were varietal differences in vapor pressure gradient; hence, one might expect varietal differences in Tr. As a consequence of higher VPDL, 1969 data exhibited

Table 10. Mean varietal transpiration rate, net photosynthesis to transpiration ratio, slope of CO_2 response curve, and diffusion resistances for three years. Experimental conditions are as given in Table 2. Significance of varietal variation given in Table 9

Variety	Year	Tr	P/Tr	S	$\sum r_o$	$\sum r_r$	$r_a + r_s$	r_a	r_s	r_{mo}	r_{mr}
Corsoy	1968	3.8	1.2	.17	4.8	4.4	2.6	1.2	1.4	2.2	1.8
	1969	4.0	0.9	.14	7.1	5.4	3.0	0.9	2.1	4.1	2.4
	1970	4.0	1.1	.17	5.3	4.4	1.1	0.8	0.3	4.2	3.3
	$\bar{x}$	3.9	1.1	.16	5.7	4.7	2.2	1.0	1.3	3.5	2.5
Amsoy	1968	3.7	1.2	.16	5.0	4.6	2.8	1.2	1.6	2.2	1.8
	1969	4.1	0.9	.15	6.4	5.0	2.9	0.9	2.0	3.4	2.1
	1970	3.7	1.1	.15	5.8	4.8	1.2	0.9	0.3	4.6	3.6
	$\bar{x}$	3.8	1.1	.15	5.7	4.8	2.3	1.0	1.3	3.4	2.5
Hawkeye	1968	3.3	1.0	.13	6.7	6.2	3.5	1.3	2.2	3.2	2.7
	1969	4.0	0.9	.14	6.5	5.2	2.8	0.9	1.9	3.7	2.4
	1970	3.5	1.1	.14	6.5	5.3	1.2	1.0	0.2	5.3	4.1
	$\bar{x}$	3.6	1.0	.14	6.6	5.6	2.5	1.1	1.4	4.1	3.1
Richland	1968	3.0	1.2	.13	6.5	6.0	3.3	1.1	2.2	3.2	2.7
	1969	3.7	0.9	.13	7.2	5.7	3.1	0.9	2.2	4.1	2.5
	1970	3.3	1.0	.13	6.9	5.6	1.2	1.0	0.2	5.7	4.4
	$\bar{x}$	3.3	1.0	.13	6.9	5.8	2.5	1.0	1.5	4.3	3.2
Provar	1970	3.7	1.2	.16	5.5	4.6	0.9	1.0	-0.1	4.6	3.7
Lindarin	1970	3.9	1.1	.16	5.5	4.6	0.9	0.9	0.0	4.6	3.6

the highest Tr. P/Tr ratios were fairly constant, with varietal differences being significant in 1970. There was a ratio of about 100:1 (w/w) of H_2O evolution to net CO_2 uptake.

The slope of the net photosynthesis versus $[CO_2]$ curve was significantly different among varieties each of the three years. The magnitude of the slope seemed very consistent among years. The reciprocal of the

Table 11. Simple correlation coefficients with transpiration and diffusion resistances, between individual observations within and among varieties for three years

Variety	Year	Tr	$r_a + r_s$			P_{320}			
		P_{320}	P_{320}	Day	R_o	r_a	r_s	r_{mo}	r_{mr}
Corsoy	1968	.51	-.74*	-.89**	-.51	—	-.66	—	-.60
	1969	.70**	-.86**	.33	-.53**	-.62**	-.87**	-.83**	-.84**
	1970	.42*	-.65**	.32	.00	-.26	—	—	-.88**
Amsoy	1968	.60	-.69*	-.59	-.72*	—	-.65*	—	-.37
	1969	.76**	-.86**	-.20	-.55**	-.68**	-.86**	-.93**	-.75**
	1970	.63**	-.60**	.30	.05	-.15	—	—	-.80**
Hawkeye	1968	.87	-.99**	-.80	-.90	—	-.96*	—	-.88
	1969	.67**	-.73**	-.37	-.60**	-.36	-.76**	-.83**	-.68**
	1970	.66**	-.70**	.04	-.28	-.51**	—	—	-.85**
Richland	1968	.67	-.83**	-.50	-.09	—	-.84**	—	-.50
	1969	.71**	-.80**	-.45*	-.67**	-.56**	-.80**	-.93**	-.85**
	1970	.71**	-.75**	-.23	-.35	-.43*	—	—	-.91**
Provar	1970	.41*	-.43*	.29	-.13	-.15	—	—	-.95**
Lindarin	1970	.52**	-.32	.34	.04	-.45*	—	—	-.83**
Among Varieties	1968	.65**	-.74**	-.41**	-.35**	—	-.71**	—	-.47**
	1969	.72**	-.82**	-.14	-.56**	-.54**	-.83**	-.76**	-.77**
	1970	.60**	-.62**	.10	-.19*	-.38**	—	—	-.89**

*Significant ($P<0.05$).

**Significant ($P<0.01$).

slope is proportional to $\sum r_r$, which is sum of resistances to molecular diffusion of CO_2 from the atmosphere beyond the boundary layer to the site of fixation. $\sum r_r$ assumes that the concentration of CO_2 at the site fixation $[CO_2]_{chl}$ is equal to the CO_2 compensation concentration. $\sum r_o$ was also calculated, which is the sum of resistances assuming $[CO_2]_{chl}$ equals 0 ppm CO_2. Estimates of the sum of resistances to diffusion of CO_2 include all physical resistances and, perhaps, some chemical resistances. They have an obvious negative correlation with net photosynthesis.

Sum of laminar and stomatal resistances is useful because it requires less experimental determinations and assumptions than r_s alone. $r_a + r_s$ was of about the same magnitude in 1968 and 1969, but it was much lower in 1970. Different wicking material was used in the psychrometers each year, and 1970 wicking seemed the most satisfactory. It is not believed that the large difference in magnitude of $r_a + r_s$ among years was entirely an artifact of experimentation. Perhaps, the higher windspeed and the plant material in 1970 were responsible, in part, for the low $r_a + r_s$.

$r_a + r_s$ had a high negative correlation with net photosynthesis (Table 11). This high correlation implies laminar and stomatal control over net photosynthesis. This control would be essentially physical in nature. $r_a + r_s$ appeared to vary over the season in an unpredictable manner. R_o was generally negatively correlated to $r_a + r_s$. These results indicate a possible physical resistance to influx and efflux of CO_2 to partially regulate photosynthesis and respiration.

Laminar resistance was estimated all three years but was statistically analyzed only in 1969 and 1970 (r_a was not analyzed in 1968 because

variation in r_a was solely a function of the experimental procedure and not the leaf). In 1969 and 1970, r_a was a function of leaf area, air temperature, and leaf temperature. To estimate r_a with large leaves in 1969 and 1970 required extrapolation beyond the experimental data obtained with wet blotters. This extrapolation was held to a minimum in 1969 by treating all leaves larger than 0.5 dm^2 as 0.5 dm^2 for prediction of r_a. This was not done in 1970 and, probably resulted in an over-estimate of r_a for large leaves. Significant differences in r_a for 1970 are believed a result of differences in leaf area.

Stomatal resistance was derived by subtraction $((r_a + r_s) - r_a)$, and hence, depended upon the accuracy of r_a estimation. For this reason, r_s values are not considered as accurate as $r_a + r_s$. Magnitude of r_s varied greatly among years with 1970 being very low. It is believed the very low r_s in 1970 is an artifact of measurement. For example, r_s for Provar was negative. The low r_s in 1970 may be, in part, a result of over-estimating r_a. Nevertheless, r_s in 1970 was still quite small, perhaps indicating an actual lower stomatal resistance to CO_2 diffusion too. Because of negative values in 1970, r_s was not analyzed for varietal differences. There were, however, varietal differences in 1968, but not in 1969. r_s was highly negatively correlated with net photosynthesis in 1968 and 1969.

Two different types of mesophyll resistances were calculated; r_{mo} represents mesophyll resistance based upon the assumption that $[CO_2]_{chl}$ equals 0 ppm, r_{mr} assumes $[CO_2]_{chl}$ equals Γ. r_{mr} was significantly different among varieties in 1968 and 1970. This resistance was higher

in 1970, perhaps as a consequence of artificially low $r_a + r_s$ values. The magnitude of r_{mr} is about 1 $sec \cdot cm^{-1}$ lower than r_{mo}. As expected, the r_{mo} and r_{mr}, are highly negatively correlated with P_{320}.

It is desirable to know what proportion of the variance in P_{320} is attributable to $r_a + r_s$ or r_{mr}. By multiple linear regression of independent variables, $r_a + r_s$ and r_{mr}, with the dependent variable, P_{320}, it was possible to estimate the proportion of sum of squares due to $r_a + r_s$ and r_{mr} independently (Table 12). $r_a + r_s$ and r_{mr} combined accounted for 81% and 91% of the variability in P_{320} in 1969 and 1970. $r_a + r_s$ accounted for 22% and 13% of the variation in P_{320} in 1969 and 1970 after the variation related to r_{mr} was removed. After the variation in P_{320} related to $r_a + r_s$ was removed, r_{mr} accounted for 15% and 53% of the variation in P_{320} in 1969 and 1970. Partial correlation coefficients indicated similar relative relationships of $r_a + r_s$ and r_{mr} with P_{320}. In 1969, $r_a + r_s$ was more negatively correlated to P_{320} (holding r_{mr} constant) than r_{mr} (Table 13), though the difference is not great. In 1970, r_{mr} was more negatively correlated to P_{320} than $r_a + r_s$. It thus seems that P_{320} was controlled more by $r_a + r_s$ in 1969 and more by r_{mr} in 1970. A similar relationship is shown for partial correlation of R_o with $r_a + r_s$ or r_{mr} holding r_{mr} or $r_a + r_s$ constant.

E. Leaf Thickness and Density-Thickness

Density-thickness, DT (weight of leaf lamina per unit leaf area), earlier was postulated a possible selection index for photosynthetic efficiency in soybean lines (22, 23). Density-thickness of the leaves

Table 12. Analysis of variance of multiple regression of laminar plus stomata resistance ($r_a + r_s$) and mesophyll resistance (r_{mr}) on net photosynthesis (P_{320}) for 1969 and 1970. $\hat{P}_{320} = b_o + b_1(r_a + r_s) + b_2(r_{mr})$

Year	Source	df	SS	R^2
1969	Due to Regression	2	4309	0.81
	Due to b_o, b_1, b_2	2	4309	0.81
	Due to b_1/b_o, b_2	1	1188	0.22
	Due to b_2/b_o, b_1	1	768	0.15
	Residual	93	1008	
	Total	95	5317	
1970	Due to Regression	2	7457	0.91
	Due to b_o, b_1, b_2	2	7457	0.91
	Due to b_1/b_o, b_2	1	1029	0.13
	Due to b_2/b_o, b_1	1	4359	0.53
	Residual	153	722	
	Total	155	8179	

Table 13. Partial correlation coefficients of net photosynthesis with diffusion resistances--96 observations in 1969 and 156 in 1970

Correlation[a]	Year	r
P_{320}, $r_a + r_s \cdot r_{mr}$	1969	-0.74
	1970	-0.79
P_{320}, $r_{mr} \cdot r_a + r_s$	1969	-0.67
	1970	-0.93
R_o, $r_a + r_s \cdot r_{mr}$	1969	-0.40
	1970	0.04
R_o, $r_{mr} \cdot r_a + r_s$	1969	-0.26
	1970	-0.69

[a]Notation: P_{320}, $r_a + r_s \cdot r_{mr}$ indicates correlation between P_{320} and $r_a + r_s$ at a single value of r_{mr}.

varied among years and varieties (Tables 14 and 15). DT was highest in 1968, perhaps a result of using the entire leaf rather than leaf punches, which were used in 1969 and 1970. The lower density-thickness of 1970 relative to 1969 must be in part a result of environmental differences between years. In general, DT was highly correlated to P_{320} for the three years, with the lowest correlation during 1969 (Table 16). Correlation of DT with P_{320} within varieties was quite variable--e.g., Corsoy in 1968 versus 1969 and 1970.

DT over the midvein, DT-V, was measured in 1969 to determine whether the DT-P_{320} correlation was partially a result of the midvein or leaf lamina. The results indicate a lower correlation for DT-V than DT with P_{320}. Thus, the correlation of DT and P_{320} is probably not a result of density or thickness of the midvein conducting tissue.

The correlation of DT and TH with P_{320} are of about the same magnitude, and the correlation of DT with TH is high, which implies that thickness, as opposed to leaf density, is the character most closely related to photosynthetic rate.

Variation in leaf net photosynthesis among years can not be entirely explained by differences in leaf morphology, as indicated by DT, TH, and leaf area. P_{320} was related to DT less in 1969 than in 1968. Leaves were larger in area and thinner in 1970 compared to 1968 and 1969. In 1969 and 1970, leaf thickness was negatively related to area, indicating the larger the leaves the thinner the leaves. Larger leaves also have a lower photosynthetic rate, although in general, this is a weak relationship.

Table 14. Mean yearly density-thickness (DT), density-thickness within midvein tissue (DT-V), lamina thickness (TH), and leaf area (Area). Experimental conditions are given in Table 2

Year	DT[a]	DT-V	TH	Area
1968	.40**	—	—	.68*
1969	.30**	.43*	82**	.69**
1970	.26**	—	73**	.79**
$\bar{x}$	.32	.43	78	.72

[a]Calculated for entire leaf in 1968 and leaf punches in 1969 and 1970.

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

Leaf size generally decreased during the season (Table 16). This was accompanied by an increase in density-thickness. Apparently a large portion of the increase in DT was accounted for by an increase in lamina thickness. Then, in general, the seasonal trends were a decrease in leaf area and an increase in thickness and P_{320}.

A partial correlation was performed between P_{320} and DT, holding TH constant (Table 17). Also P_{320} was correlated with TH, holding DT constant. These partial correlations indicate lower correlations than simple correlations because of their common association (DT is correlated with TH). In 1970, DT seems to be more related to P_{320} than TH is related to P_{320}, though the difference is small. DT and TH in 1969 were similarly correlated with P_{320}. The partial correlation of P_{320} and DT, holding TH and area constant was greater than when only TH was held constant, though the difference is small. Since DT and P_{320} are associated with seasonal

Table 15. Mean varietal density-thickness, density-thickness within midvein tissue, lamina thickness, and leaf area. Experimental conditions are given in Table 2. Significance of varietal variation given in Table 14

Variety	Year	DT	DT-V	TH	Area
Corsoy	1968	.46	—	—	.61
	1969	.31	.42	88	.58
	1970	.29	—	81	.63
	$\bar{x}$	.35	.42	85	.61
Amsoy	1968	.45	—	—	.68
	1969	.32	.46	85	.68
	1970	.27	—	74	.72
	$\bar{x}$	.35	.46	80	.69
Hawkeye	1968	.38	—	—	.70
	1969	.29	.43	79	.74
	1970	.25	—	72	.83
	$\bar{x}$	.31	.43	76	.76
Richland	1968	.35	—	—	.69
	1969	.29	.43	75	.74
	1970	.23	—	65	.86
	$\bar{x}$	.29	.43	70	.76
Provar	1970	.27	—	73	.90
Lindarin	1970	.25	—	73	.80

trends (Days after June 30), P_{320} and DT were correlated holding Day constant. There was a lower correlation between P_{320} and DT holding Day constant compared to the simple correlation of P_{320} and DT. The correlations were not much smaller, however, indicating their common association with Day was not too great.

Since DT and TH were earlier suggested as possible selection indexes for P_{320} in lines of soybeans, genetic correlation coefficients (r_g) were

Table 16. Simple correlation coefficients with density-thickness, leaf thickness, and leaf area between individual observations within and among varieties for three years

Variety	Year	P_{320}				Area		DT	DAY		
		DT	DT-V	TH	Area	DT	TH	TH	DT	TH	Area
Corsoy	1968	.85**	—	—	—	—	—	—	.91**	—	—
	1969	.08	-.16	.11	.17	-.63**	-.74**	.89**	.77**	.85**	-.90**
	1970	.05	—	-.00	-.05	-.61**	-.41*	.66**	.73**	.46*	-.88**
Amsoy	1968	.81**	—	—	—	—	—	—	.69**	—	—
	1969	.36	.29	.48*	-.32	-.81**	-.76**	.87**	.84**	.85**	-.93**
	1970	.39*	—	.25	-.03	-.47*	-.66**	.72**	.44*	.52**	-.88**
Hawkeye	1968	.65**	—	—	—	—	—	—	.74**	—	—
	1969	.50*	.48*	.31	-.15	-.52**	-.53**	.73**	.66**	.58**	-.90**
	1970	.66**	—	.52**	-.23	-.14	.26	.76**	.31	-.10	-.83**
Richland	1968	.56*	—	—	—	—	—	—	.75**	—	—
	1969	.70**	.60**	.79**	-.49*	-.55**	-.62**	.89**	.75**	.77**	-.81**
	1970	.66**	—	.78**	-.13	-.17	.01	.83**	.31	.20	-.81**
Provar	1970	.77**	—	.70**	-.28	-.47*	-.24	.80**	.44*	.24	-.85**
Lindarin	1970	.54**	—	.26	-.31	-.34	-.11	.77**	.17	-.08	-.85**
Among	1968	.71**	—	—	—	—	—	—	.68**	—	—
Varieties	1969	.43**	.31**	.43**	-.20	-.62**	-.69**	.85**	.72**	.68**	-.84**
	1970	.60**	—	.58**	-.23**	-.42**	-.39**	.79**	.35**	.15	-.74**

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 17. Partial correlation coefficients of net photosynthesis with density-thickness and thickness--96 observations in 1969 and 156 in 1970

Correlation	Year	r
P_{320}, DT•TH	1969	0.14
	1970	0.28
P_{320}, TH•DT	1969	0.14
	1970	0.22
P_{320}, DT•TH, Area	1969	0.15
	1970	0.29
P_{320}, Day•DT	1969	-0.05
	1970	0.01
P_{320}, DT•Day	1969	0.34
	1970	0.57

Table 18. Genetic (r_g) and simple (r) correlation coefficients of net photosynthesis (P_{320}) with leaf density-thickness (DT) and leaf thickness (TH). Correlations are for individual leaf observations

Year	r_g		r	
	DT	TH	DT	TH
1968	0.93	—	0.71	—
1969	1.23	1.18	0.43	0.43
1970	0.96	0.92	0.60	0.58

calculated (Table 18). The reader is reminded that the r_g's are estimates and hence, it is possible to have a correlation larger than 1.00. The genetic correlations are quite high between P_{320} and DT and TH. In 1969, the genetic correlations were highest and simple correlations were lowest of all three years.

F. Anatomy

One of the purposes of the research was to examine the internal anatomy of the soybean leaves, and to study the relationships between anatomy and net photosynthetic rate. Four leaves were examined per variety each year in 1969 and 1970. These leaves were measured about a week apart. Thus, the data will give some indication of varietal, yearly, and seasonal variation in anatomy.

1. Stomatal apertures and density

Stomatal apertures and densities were measured from epidermal impressions of the leaf surfaces. It is possible that the aperture estimates are subject to some error, because of stomatal closure while the silicone rubber was drying, and/or the silicone rubber does not give an accurate impression of the smallest portion of the stomatal pore. It is believed that these aperture measurements can give a rough idea of the relative openings, however. The stomatal densities are accurate, but of limited sample area.

There was little varietal difference in the various aperture measurements (Table 19). Width of stomata on adaxial surface, W_{ad}, only was significant in 1969, but not in 1970. The stomata were wider in 1969 than in 1970. Stomata on the abaxial surface were wider than those on the adaxial surface of the leaf. Felch (32) also reports smaller apertures on the adaxial surface of Provar and Hark varieties. Lengths of stomata apertures were about the same both years and on both surfaces and were not significantly different among varieties.

Table 19. Mean net photosynthetic rate (P_{320}), width of stomata on adaxial and abaxial surfaces (W_{ad} and W_{ab}), length of stomata on adaxial and abaxial surfaces (L_{ad} and L_{ab}), and density of stomata on adaxial and abaxial surfaces (N_{ad} and N_{ab}) of four leaves per variety per year

Variety	Year	P_{320}	W_{ad}	W_{ab}	L_{ad}	L_{ab}	N_{ad}	N_{ab}
Corsoy	1969	40.1	6.3	8.2	13.5	12.6	209	380
	1970	45.2	2.9	4.8	12.9	10.1	286	550
	$\bar{x}$	42.7	4.6	6.5	13.2	11.4	248	465
Amsoy	1969	39.8	4.2	6.3	10.7	13.1	179	442
	1970	43.2	1.9	4.3	13.5	11.0	217	418
	$\bar{x}$	41.5	3.1	5.3	12.1	12.1	198	430
Hawkeye	1969	33.8	2.7	7.2	10.6	12.3	229	380
	1970	37.9	1.8	3.7	12.6	9.1	294	488
	$\bar{x}$	35.9	2.3	5.5	11.6	10.7	262	434
Richland	1969	30.5	1.7	6.1	6.3	11.6	97	333
	1970	34.3	3.0	3.6	11.2	10.1	255	441
	$\bar{x}$	32.4	2.4	4.9	8.8	10.9	176	387
Provar	1970	44.4	3.5	6.6	13.8	11.4	294	681
Lindarin	1970	39.6	3.5	4.7	12.6	11.0	271	503
Mean	1969	36.1	3.7+	7.0	10.3	12.4	179**	384+
	1970	40.8	2.8	4.6	12.8	10.5	270	514**
	$\bar{x}$	38.5	3.3	5.8	11.6	11.5	225	449

+Significant varietal variation ($P<0.10$).

**Significant varietal variation ($P<0.01$).

Differences among varieties in stomatal densities on the adaxial, N_{ad}, were significant in 1969, and both years varietal differences on the abaxial surface, N_{ab}, were significant. There were approximately twice as many stomata on the abaxial surface. Felch (32) reports three times as many stomata on the abaxial surface of soybeans. There were also fewer stomata per unit leaf surface in 1969 compared to 1970.

Net photosynthesis was correlated with some stomatal parameters, but none were consistently highly correlated (Table 20). In general, P_{320} was positively correlated with W_{ad}, L_{ad}, and N_{ab}. There was, however, much variability between years and among varieties. Laminar and stomatal resistance, $r_a + r_s$, was, in general, negatively correlated with all stomatal parameters.

Seasonal trends (Table 20) seemed quite variable among years and varieties. Some stomatal attributes in some varieties seemed highly correlated with days after June 30. However, the correlations among varieties and years were small and not significant.

2. Cellular and tissue dimensions

Thickness of the leaf clearings, T_c, was examined microscopically to compare with fresh-leaf thickness measurements, T_m. They were not expected to agree perfectly, because of chemical alteration of the clearings and sampling errors. They were, however, quite similar in magnitude (Table 21), which indicates that the chemical may not have altered the cellular dimension too greatly. The soybean leaves were about 200 μ thick. Table 21 gives the varietal and yearly means of leaf thickness and other dimensions. Varieties differed, significantly, in T_m and T_c both years.

Table 20. Simple correlation coefficients with width of stomata, length of stomata, and density of stomata between individual observations within and among varieties for two years, and among varieties and years[a]

Variety	Year	P320						$r_a + r_s$		
		W_{ad}	W_{ab}	L_{ad}	L_{ab}	N_{ad}	N_{ab}	W_{ad}	W_{ab}	L_{ad}
Corsoy	1969	.95*	-.30	.63	-.72	.34	.80	-.55	-.48	-.74
	1970	-.29	-.45	.71	-.71	-.90	.65	-.58	-.66	.83
Amsoy	1969	.87	.85	.96*	.04	-.23	-.86	-.79	-.90	-.98*
	1970	.61	.67	.31	.90	-.55	-.01	-.46	.53	.94
Hawkeye	1969	.99**	.57	.76	.38	-.48	-.46	-.00	-.51	-.42
	1970	.20	-.50	-.15	-.39	.91	.42	-.01	.78	.16
Richland	1969	.51	-.32	.98*	.17	.69	.17	-.44	.30	-.80
	1970	-.06	.55	-.16	-.24	.19	-.16	-.32	-.85	.15
Provar	1970	.40	.85	-.08	1.00**	-.18	.36	-.68	-.80	.68
Lindarin	1970	.74	.70	.91	-.13	-.49	.23	-.92	-.91	-.82
Among Varieties	1969	.82**	.22	.77**	.09	.29	.35	-.51*	-.25	-.68**
	1970	.21	.50*	.37	.24	-.17	.40*	-.31	-.57**	-.09
Among Varieties and Years		.46**	-.01	.65**	-.12	.24	.43*	-.05	.44*	-.54**

[a]Common simple correlations over the four common varieties for 1969 and 1970.

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 20. (Continued)

Variety	Year	$r_a + r_s$			DAY					
		L_{ab}	N_{ad}	N_{ab}	W_{ad}	W_{ab}	L_{ad}	L_{ab}	N_{ad}	N_{ab}
Corsoy	1969	-.41	.28	.36	.29	-.71	-.09	-.94	.57	.95*
	1970	-.02	-.79	.10	.17	.02	.23	-.66	-.53	.51
Amsoy	1969	-.07	-.15	.77	.17	.01	.29	-.65	-.86	-.81
	1970	.46	-.04	-.71	-.26	.27	1.00**	.31	-.36	-.89
Hawkeye	1969	-.85	-.51	.75	.57	.99**	.38	.80	.40	-.34
	1970	.76	-.80	-.72	-.95*	-.32	.96*	-.27	.00	.41
Richland	1969	.18	-.53	.41	.33	-.41	.89	.21	.77	.50
	1970	-.12	-.01	-.25	.05	.39	-.67	-.01	.72	-.08
Provar	1970	-.81	-.38	-.81	-.61	-.03	.97*	.09	-.77	-.73
Lindarin	1970	-.18	.55	-.57	-.85	-.68	-.23	.12	-.19	-.48
Among Varieties	1969	-.26	-.40	-.11	.27	-.01	.41	-.16	.07	-.00
	1970	.04	-.14	-.50*	-.22	-.04	.32	.01	-.10	-.01
Among Varieties and Years		.48**	-.65**	-.49**	.16	.22	.27	.10	-.20	-.17

Table 21. Mean leaf thickness measured by mechanical micrometer (T_m), leaf thickness from leaf clearings by microscope micrometer (T_c), thickness of upper palisade layer (L_U), thickness of lower palisade layer (L_L), thickness of paraveinal layer (L_{PV}), thickness of spongy mesophyll layer (L_S), diameter of upper palisade cells (D_U), and diameter of lower palisade cells (D_L) of four leaves per variety per year

Variety	Year	T_m	T_c	L_U	L_L	L_{PV}	L_S	D_U	D_L
Corsoy	1969	225	207	54.6	42.3	58.0[a]		9.5	9.5
	1970	198	290	37.0	35.3	14.8	41.3	7.8	9.1
	$\bar{x}$	212	249	45.8	38.8			8.7	9.3
Amsoy	1969	204	188	45.2	41.1	57.0		9.1	9.2
	1970	197	276	37.5	38.9	11.5	46.0	8.3	9.4
	$\bar{x}$	201	232	41.4	40.0			8.7	9.3
Hawkeye	1969	198	156	42.5	37.8	49.0		7.9	8.5
	1970	183	213	38.0	39.0	10.4	34.2	8.3	9.3
	$\bar{x}$	191	185	40.3	38.4			8.1	8.9
Richland	1969	172	142	42.9	33.9	44.0		8.4	9.4
	1970	164	195	32.2	33.2	9.0	27.9	7.9	9.3
	$\bar{x}$	168	169	37.6	33.6			8.2	9.4
Provar	1970	185	277	36.0	35.6	13.5	23.1	7.5	10.0
Lindarin	1970	181	226	34.4	34.3	12.7	30.5	7.2	8.2
Mean	1969	200*	173**	46.3	38.8	52.0		8.7+	9.2
	1970	185*	246*	35.9	36.6	12.0	33.8*	7.8	9.2+
	$\bar{x}$	193	210	41.1	37.7			8.3	9.2

[a]Thickness of paraveinal and spongy mesophyll layers combined.

+Significant varietal variation ($P<0.10$).

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

Among varieties and years, T_m and T_c were both highly correlated with P_{320} (Table 22). T_m and T_c also increased during the season in 1969, but not in 1970. Partitioning leaf thickness into various tissue thicknesses gives an estimate of relative importance of specific tissues. The upper palisade cells, L_U, were longer in 1969 than 1970. Upper palisade cells were also slightly longer than the lower palisade cells, L_L, in 1969, but not in 1970. The longer upper palisade cells were also of smaller diameter, D_U, than lower palisade cells, D_L. Thickness of the paraveinal layer, L_{PV}, is about 1/3 to 1/4 that of the other tissue layers. In 1969, paraveinal and spongy mesophyll thickness, L_S, were combined in measurement. Of the specific tissue dimensions, significant varietal differences were exhibited only for L_S in 1970.

Correlations of the various tissue dimensions with P_{320} are presented in Table 22. Thickness of all tissue layers in the leaf were positively correlated with P_{320}, indicating that each may contribute to the variation in leaf thickness. In 1969, all tissue thicknesses increased during the season; however, this was not true in 1970. In 1970, only the paraveinal layer increased during the season. Mesophyll resistance was negatively correlated with all tissue thicknesses.

Diameters of palisade cells were poorly correlated with net photosynthesis (Table 22). The cell diameters did not show any strong trends during the season. They were not highly correlated to r_{mr} either, but there was a trend toward a negative correlation with r_{mr}.

Table 22. Simple correlation coefficients with leaf thickness, thickness of various tissues within the leaf, and diameter of palisade cells between individual observations within and among varieties for two years, and among varieties and years

Variety	Year	P_{320}							
		T_m	T_c	L_U	L_L	L_{PV}	L_S	D_U	D_L
Corsoy	1969	.77	.57	.64	.46	.55[a]		.23	.80
	1970	.25	.72	-.25	-.39	.43	.56	.10	-.48
Amsoy	1969	.88	.59	.81	.53	.35		-.44	-.18
	1970	.90	.22	.91	.78	-.97*	.64	-.18	.77
Hawkeye	1969	.93	.92	.92	.79	.86		-.22	.04
	1970	.58	.19	.73	.78	-.46	-.20	.61	.20
Richland	1969	.83	.28	.85	.83	.35		-.67	-.56
	1970	.21	.38	-.40	-.13	.57	.77	-.40	.52
Provar	1970	.89	.94	.56	.65	-.01	.88	.89	.99**
Lindarin	1970	.14	.37	.47	.39	.47	.61	.10	.25
All Varieties	1969	.82**	.68**	.72**	.69**	.61*		.09	.01
	1970	.72**	.72**	.45*	.39	.39	.48*	.01	.26
Among Varieties and Years		.65**	.70**	.36*	.49**	.36*		-.09	.04

[a]Combined thickness of paraveinal and spongy mesophyll layers.

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 22. (Continued)

Variety	Year	DAY							
		T_m	T_o	L_U	L_L	L_{PV}	L_S	D_U	D_L
Corsoy	1969	.94	1.00**	.97*	.99**	1.00**[a]		.47	-.05
	1970	.56	.33	-.50	-.70	.74	.85	-.35	-.87
Amsoy	1969	.78	.99**	.81	.77	.83		.29	.73
	1970	.49	-.83	.35	.84	-.28	.34	.86	.71
Hawkeye	1969	.27	.87	.25	.22	.29		.38	.56
	1970	-.34	-.87	.02	-.30	.46	.29	-.20	.49
Richland	1969	.96*	.58	.95*	.96*	.65		-.37	-.44
	1970	.73	.62	-.75	.24	.93	.28	-.84	.27
Provar	1970	.99**	.99**	.86	.91	.39	1.00**	.99**	.92
Lindarin	1970	-.84	-.96*	-.96*	-.85	-.75	-.96*	-.85	-.92
All Varieties	1969	.61*	.62**	.68**	.70**	.62**		.04	.16
	1970	.15	-.18	-.09	.09	.31	.19	-.24	-.05
Among Varieties and Years		.49**	-.09	.50**	.50**	.56**		.07	.09

Table 22. (Continued)

Variety	Year	r_{mr}							
		T_m	T_c	L_U	L_L	L_{PV}	L_S	D_U	D_L
Corsoy	1969	-.90	-.79	-.86	-.72		-.78	-.43	-.56
	1970	-.13	-.80	.15	.20	-.28	-.41	-.25	.28
Amsoy	1969	-.96*	-.81	-.92	-.70		-.59	.19	-.13
	1970	-.96*	-.01	-.85	-.89	.91	-.73	-.03	-.80
Hawkeye	1969	-.94	-.90	-.93	-.83		-.90	.19	.04
	1970	-.88	-.48	-.69	-.73	.89	-.92	-.86	-.71
Richland	1969	-.55	-.58	-.47	-.59		-.93	-.21	.52
	1970	-.44	-.74	.34	-.31	-.75	-.62	.50	-.70
Provar	1970	-.93	-.98*	-.66	-.76	-.10	-.94	-.95*	-.99**
Lindarin	1970	-.72	-.39	-.55	.05	.31	-.05	-.46	-.54
All Varieties	1969	-.71**	-.71**	-.75**	-.70**		-.79**	-.26	-.03
	1970	-.79**	-.71**	-.47*	-.44*	-.33	-.59**	-.11	-.29
Among Varieties and Years		-.68**	.03	-.73**	-.53**		-.85**	-.39*	-.02

3. Exposed surface area

Internal exposed surface area of the cell walls also was estimated for the different tissues within the leaf (Table 23). In 1969, as noted before, the paraveinal and spongy layers were thought to be only one layer, the spongy mesophyll, and hence, were combined. There were significant varietal differences in spongy parenchyma exposed surface area, S_S. Upper palisade exposed surface area, S_U, was significantly different among varieties in 1970, but not in 1969. In neither year was surface area of the lower palisade layer, S_L, different among varieties. Total internal cellular surface area, S_T, was not different among varieties. Ratio of surface area of each cellular layer to total internal surface area differed among varieties in 1970, but only proportion of upper palisade differed in 1969. In general, there seems a correlation between photosynthetic rate and surface area of the various tissues separately and in total, though the relationships were poorer and not statistically significant in 1970 (Table 24). The correlation of exposed surface area with r_{mf} was of about the same magnitude as with P_{320}.

The total internal exposed surface area, S_T, was higher in 1969 than in 1970. S_U was largely responsible for this variation. Total exposed surface area increased during the season both years, but more markedly in 1969.

Each palisade layer contributed about 1/3 of the exposed surface area and the spongy and paraveinal together contributed the other 1/3 of the total area. In 1969, the upper palisade contributed the most, whereas in 1970, the lower palisade area was slightly greater. Table 23 also

Table 23. Mean exposed surface area of upper palisade tissue (S_U), lower palisade tissue (S_L), paraveinal tissue (S_{PV}), spongy mesophyll tissue (S_S), total exposed surface area within the leaf (S_T), proportion of exposed surface area of each tissue to the total and ratio of exposed surface area to external surface area (S_E) of four leaves per variety per year

Variety	Year	S_U	S_L	S_{PV}	S_S	S_T	$\frac{S_U}{S_T}$	$\frac{S_L}{S_T}$
Corsoy	1969	11.8	7.1		6.2	25.1	.47	.28
	1970	5.2	5.8	1.1	4.7	16.8	.31	.35
	$\bar{x}$	8.5	6.5			21.0	.39	.32
Amsoy	1969	9.9	7.7		6.3	23.9	.42	.32
	1970	6.6	7.2	0.8	6.2	20.8	.31	.34
	$\bar{x}$	8.3	7.5			22.4	.37	.33
Hawkeye	1969	9.8	6.5		5.1	21.4	.46	.31
	1970	7.1	7.4	0.7	4.2	19.5	.36	.38
	$\bar{x}$	8.5	7.0			20.5	.41	.35
Richland	1969	9.2	6.1		3.0	18.1	.51	.32
	1970	5.1	6.3	0.7	3.4	15.5	.33	.40
	$\bar{x}$	7.2	6.2			16.8	.42	.36
Provar	1970	4.4	6.3	1.0	2.3	14.0	.32	.45
Lindarin	1970	6.7	6.0	1.0	3.6	17.3	.38	.35
Means	1969	10.2	6.9		5.2*	20.0	.47*	.31
	1970	5.9+	6.5	0.9	4.1*	17.3	.34+	.38*
	$\bar{x}$	8.1	6.7			18.7	.41	.35

+Significant varietal variation ($P<0.10$).

*Significant varietal variation ($P<0.05$).

Table 23. (Continued)

Variety	Year	$\frac{S_{PV}}{S_T}$	$\frac{S_S}{S_T}$	$\frac{S_U}{S_E}$	$\frac{S_L}{S_E}$	$\frac{S_{PV}}{S_E}$	$\frac{S_S}{S_E}$	$\frac{S_T}{S_E}$
Corsoy	1969	.25		4.9	2.9	2.6		10.4
	1970	.06	.27	2.2	2.4	.45	2.0	6.9
	$\bar{x}$			3.5	2.7			8.7
Amsoy	1969	.27		4.1	3.2	2.6		9.9
	1970	.04	.29	2.7	3.0	.33	2.6	8.6
	$\bar{x}$			3.4	3.1			9.2
Hawkeye	1969	.24		4.0	2.7	2.1		8.8
	1970	.04	.22	2.9	3.1	.30	1.7	8.1
	$\bar{x}$			3.5	2.9			8.5
Richland	1969	.17		3.8	2.5	1.2		7.5
	1970	.04	.22	2.1	2.6	.27	1.4	6.4
	$\bar{x}$			2.9	2.6			6.9
Provar	1970	.08	.16	1.8	2.6	.43	1.0	5.8
Lindarin	1970	.05	.21	2.8	2.5	.40	1.5	7.1
Means	1969	.23		4.2	2.8	2.1*		9.2
	1970	.05*	.23*	2.4+	2.7	.36	1.7*	7.2
	$\bar{x}$			3.3	2.8			8.2

presents the ratios of internal exposed surface area of the various tissues to external surface area (both sides) of the sample. There was approximately eight times as much internal exposed surface area as external surface area. Varieties were not significantly different in this character. Significant varietal differences in ratio of upper palisade (1970 only) and spongy mesophyll area to external surface were demonstrated.

Table 24. Simple correlation coefficients with exposed surface area of various tissue layers between individual observations within and among varieties for two years, and among varieties and years

Variety	Year	P_{320}				
		S_U	S_L	S_{PV}	S_S	S_T
Corsoy	1969	.76	.38	.68		.67
	1970	.62	-.46	.25	.54	.44
Amsoy	1969	.76	.62	-.88		-.13
	1970	.89	.95*	-1.00**	.72	.91
Hawkeye	1969	.86	.65	-.03		.69
	1970	.74	.72	-.35	-.46	.63
Richland	1969	.66	.77	.66		.78
	1970	-.84	.30	.56	.56	.33
Provar	1970	.38	.51	.24	.29	.43
Lindarin	1970	.19	.28	.44	.77	.37
Among Varieties	1969	.69**	.64**	.41		.73**
	1970	.12	.25	.35	.35	.32
Among Varieties and Years		.17	.44*	.43*		.44*

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 24. (Continued)

Variety	Year	r_{mr}					DAY
		S_U	S_L	S_{PV}	S_S	S_T	S_T
Corsoy	1969	-.93	-.64	-.82		-.87	.94
	1970	-.42	.35	-.07	-.36	-.27	.79
Amsoy	1969	-.70	-.69	.89		.11	-.15
	1970	-.92	-1.00**	.99**	-.82	-.98*	.65
Hawkeye	1969	-.83	-.59	.07		-.64	-.74
	1970	.15	-.76	.80	-.52	-.49	.31
Richland	1969	-.20	-.50	-.66		-.42	.90
	1970	.56	-.68	-.72	-.52	-.66	.33
Provar	1970	-.50	-.64	-.34	-.43	-.56	.76
Lindarin	1970	-.60	-.18	.51	-.08	-.32	-.96*
Among Varieties	1969	-.68**	-.60*	-.21		-.64**	.54*
	1970	-.19	-.34	-.25	-.47*	-.43*	.25
Among Varieties and Years		-.75**	-.38*	-.41*		-.68**	.55**

4. Intercellular space

Varietal differences in total intercellular space were present in 1970 (Table 25). The variation was largely a result of upper palisade layer and spongy mesophyll intercellular space variation. In general, the volume of intercellular space was correlated with P_{320}; the correlation was highest in 1969 (Table 26). The sum of laminar and stomata resistance was weakly negatively correlated with volume of intercellular space. Mesophyll resistance was more highly negatively correlated with V_T than $r_a + r_s$. Volume of intercellular space was higher in 1969 compared to 1970. This was largely a result of more intercellular space in the palisade layers in 1969. Intercellular space increased during the season of 1969, but not in 1970.

Leaves in 1969 were about 40% intercellular space, but only 25% in 1970 (Table 25); these estimates are minimal since V_{ET} was estimated by $1.21 \times 10^4\ \mu^2 \cdot T_c$ rather than $V_{EU} + V_{EL} + V_{EPV} + V_{ES}$. Elizabeth et al. (27) have found Merit soybeans, grown under 27.5/22.5°C (day/night) and high light (220 W/m^2), to exhibit 50% intercellular space. The palisade layers had a higher portion of intercellular space in 1969—i.e., they were probably less dense in 1969. The spongy mesophyll seemed to have the highest percent of intercellular space. All the tissues, in fact, possessed an appreciable amount of intercellular space.

5. Cellular volume

Total cellular volume of the leaf sample, V_{CT}, was larger in 1969 than 1970 (Table 27). There was no significant varietal variation in

Table 25. Mean volume of intercellular space within the upper palisade tissue (V_U), lower palisade tissue (V_L), paraveinal tissue (V_{PV}), spongy mesophyll tissue (V_S), total internal intercellular space within the leaves (V_T), and ratio of volume of intercellular space to total volume for various tissues (V_U/V_{EU}, V_L/V_{EL}, V_{PV}/V_{EPV}, V_S/V_{ES}, V_T/V_{ET}) of four leaves per variety per year

Variety	Year	V_U	V_L	V_{PV}	V_S	V_T
Corsoy	1969	31.4	31.0		30.6	93.0
	1970	17.5	22.8	8.6	32.2	81.2
	$\bar{x}$	24.5	26.9			87.1
Amsoy	1969	25.6	27.8		29.2	82.4
	1970	19.4	24.0	5.8	34.7	84.0
	$\bar{x}$	22.5	25.9			83.2
Hawkeye	1969	25.4	26.5		27.4	79.2
	1970	19.3	20.7	5.5	26.2	71.5
	$\bar{x}$	22.4	23.6			75.4
Richland	1969	27.2	22.8		24.1	74.0
	1970	15.2	18.0	5.1	22.6	61.0
	$\bar{x}$	20.2	20.4			67.5
Provar	1970	11.4	18.0	6.6	19.5	55.6
Lindarin	1970	20.7	22.2	7.1	25.9	75.9
Means	1969	27.4	27.0		27.8	82.2
	1970	17.3**	21.0	6.5	26.9+	71.5*
	$\bar{x}$	22.4	24.0			76.9

+Significant varietal variation ($P<0.10$).

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

Table 25. (Continued)

Variety	Year	$\frac{V_U}{V_{EU}}$	$\frac{V_L}{V_{EL}}$	$\frac{V_{PV}}{V_{EPV}}$	$\frac{V_S}{V_{ES}}$	$\frac{V_T}{V_{ET}}$
Corsoy	1969	.48	.61		.44	.37
	1970	.39	.54	.49	.66	.23
	$\bar{x}$	.44	.58			.30
Amsoy	1969	.46	.56		.42	.36
	1970	.43	.52	.43	.62	.25
	$\bar{x}$	.45	.54			.31
Hawkeye	1969	.50	.57		.46	.43
	1970	.42	.44	.43	.63	.28
	$\bar{x}$	.46	.51			.36
Richland	1969	.52	.57		.45	.44
	1970	.39	.45	.49	.67	.26
	$\bar{x}$	.46	.51			.35
Provar	1970	.27	.43	.41	.70	.17
Lindarin	1970	.50	.54	.46	.70	.28
Means	1969	.49	.58		.44	.40
	1970	.40**	.49	.45	.66	.25*
	$\bar{x}$	.45	.54			.33

total leaf cellular volume. Volume of cells in the spongy layer was significantly different among varieties in 1970, but other layers were not different among varieties either year. Each palisade layer comprised about 1/3 of the total cellular volume, and the paraveinal and spongy layers the remaining 1/3 of the cellular volume. Elizabeth et al. (27) found that Merit soybeans grown under 27.5/22.5°C (day/night) and high light (220 W/m^2), consisted of 3-4 layers of palisade cells, and 2/3 of

Table 26. Simple correlation coefficients with volume of intercellular space within various tissue layers between individual observations within and among varieties for two years and among varieties and years

Variety	Year	P_{320}				
		V_U	V_L	V_{PV}	V_S	V_T
Corsoy	1969	.53	.36		.78	.61
	1970	.54	.88	.34	.58	.82
Amsoy	1969	.78	.23		.58	.61
	1970	.96*	.26	-.85	.53	.70
Hawkeye	1969	.41	.09		.67	.31
	1970	.48	.56	-.51	-.06	.37
Richland	1969	.98*	.57		.38	.92
	1970	-.73	-.73	.33	.90	-.31
Provar	1970	.63	.53	-.66	.94	.81
Lindarin	1970	.98*	.38	.60	.57	.65
Among Varieties	1969	.64**	.40		.65**	.64**
	1970	.09	.37	.27	.50*	.45*
Among Varieties and Years		.21	.26		.65**	.56**

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 26. (Continued)

Variety	Year	$r_a + r_s$					r_{mr}	DAY
		V_U	V_L	V_{PV}	V_S	V_T	V_T	V_T
Corsoy	1969	.66	.71		.39	.59	-.82	1.00**
	1970	.69	.12	-.58	-.30	-.12	-.69	.96*
Amsoy	1969	-.56	.16		-.21	-.26	-.81	.92
	1970	.09	.99**	-.37	.57	.77	-.81	.57
Hawkeye	1969	-.28	-.49		-.49	-.45	-.39	-.15
	1970	-.34	-.25	.01	.55	.07	-.81	-.69
Richland	1969	-.87	-.20		.22	-.52	-.73	.96*
	1970	.38	.50	-.28	-.97*	-.10	.60	-.21
Provar	1970	-.17	.11	.97*	-.53	-.27	-.77	.75
Lindarin	1970	-.93	-.68	-.84	-.77	-.87	.32	-.80
Among Varieties	1969	-.46	-.20		-.09	-.29	-.69**	.62**
	1970	.03	-.10	-.29	-.01	-.07	-.49*	.02
Among Varieties and Years		.34	.27		-.08	.07	-.65**	.47**

the cellular volume was palisade tissue while 1/3 was spongy mesophyll. In general, total cellular volume was positively correlated with net photosynthesis (Table 28). Mesophyll resistance was negatively correlated with cellular volume in about the same degree. Each tissue layer seemed about equally correlated to P_{320} and r_{mr}. Total cellular volume increased during the season in 1969, but not in 1970. V_{CT} among varieties was only weakly, negatively correlated to $r_a + r_s$ (correlations among varieties and years involve variation of $r_a + r_s$ between years).

6. Surface to volume ratio

Exposed cellular surface area to cellular volume of the various tissues within the leaves also was calculated. The total surface/volume,

Table 27. Mean cellular volume within the upper palisade tissue (V_{CU}), lower palisade tissue (V_{CL}), paraveinal tissue (V_{CPV}), spongy mesophyll tissue (V_{CS}), and total internal cellular volume within the leaves (V_{CT}) of four leaves per variety per year

Variety	Year	V_{CU}	V_{CL}	V_{CPV}	V_{CS}	V_{CT}[a]
Corsoy	1969	34.8	20.2	39.5		94.5
	1970	27.2	19.9	9.4	17.8	74.2
	$\bar{x}$	31.0	20.1			84.4
Amsoy	1969	29.2	21.9	40.0		91.1
	1970	26.0	23.0	8.0	20.9	77.9
	$\bar{x}$	27.6	22.5			84.5
Hawkeye	1969	26.1	19.3	32.3		77.7
	1970	26.7	26.5	7.1	15.2	75.5
	$\bar{x}$	26.4	22.9			76.6
Richland	1969	24.7	18.3	28.9		71.9
	1970	23.8	22.2	5.7	11.1	62.8
	$\bar{x}$	24.3	20.3			67.4
Provar	1970	32.2	25.1	9.7	8.4	75.4
Lindarin	1970	20.9	19.3	8.3	11.0	59.4
Means	1969	28.7	19.9	35.2		83.8
	1970	26.1	22.7	8.0	14.1*	70.9
	$\bar{x}$	27.4	21.3			77.4

[a]V_{CT} for 1969 was calculated from varietal means and not analyzed statistically.

*Significant varietal variation ($P<0.05$).

Table 28. Simple correlation coefficients with cellular volume within various tissue layers between individual observations within and among varieties for two years and among varieties and years

Variety	Year	P_{320}				
		V_{CU}	V_{CL}	V_{CPV}	V_{CS}	V_{CT}[a]
Corsoy	1969	.71	.46	.30		.53
	1970	-.54	-.74	.47	.52	-.06
Amsoy	1969	.38	.81	-.25		.53
	1970	.86	.95*	-.84	.80	.96*
Hawkeye	1969	.87	.47	.91		.53
	1970	.33	.62	-.43	-.44	.27
Richland	1969	.09	.66	.30		-.11
	1970	.11	.24	.66	.50	.40
Provar	1970	.43	.59	.45	.32	.49
Lindarin	1970	.06	.25	.11	.70	.29
Among Varieties	1969	.59*	.53*	.52*		.54*
	1970	.40*	.21	.42*	.40*	.49*
Among Varieties and Years		.40*	.36*	.09		.12

[a] V_{CT} for 1969 employed T_c in calculations rather than $V_{CU} + V_{CL} + V_{CPV} + V_{CS}$ as in 1970.

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 28. (Continued)

Variety	Year	r_{mr}					DAY	$r_a + r_s$
		V_{CU}	V_{CL}	V_{CPV}	V_{CS}	V_{CT}^a	V_{CT}^a	V_{CT}^a
Corsoy	1969	-.90	-.69		-.58	-.76	1.00**	.67
	1970	.42	.58	-.31	-.33	.17	.29	-.81
Amsoy	1969	-.36	-.76		-.06	-.75	.99**	-.31
	1970	-.81	-.99**	.76	-.87	-1.00**	.57	.59
Hawkeye	1969	-.83	-.39		-.92	-.46	.79	.24
	1970	-.45	-.58	.86	-.78	-.59	.56	.06
Richland	1969	-.56	-.31		-.79	-.31	.21	.44
	1970	-.31	-.62	-.88	-.47	-.65	.24	-.01
Provar	1970	-.56	-.71	-.57	-.46	-.62	.78	-.43
Lindarin	1970	-.73	-.52	-.30	-.08	-.51	-.94	-.43
Among Varieties	1969	-.75**	-.48		-.65**	-.54*	.46	-.14
	1970	-.42*	-.26	-.39	-.52**	-.56**	.09	-.16
Among Varieties and Years		-.55**	-.04		-.84**	-.75**	.50**	.54**

S_T/V_{CT}, of the cells was slightly higher in 1969. There were varietal differences in 1970 in S_T/V_{CT}, S_U/V_{CU}, and S_S/V_{CS} (Table 29). Provar had the lowest surface to volume ratio of the cells. Paraveinal tissue exhibited the lowest surface to volume ratios.

Surface to volume ratios were not consistently correlated with P_{320} (Table 30) or r_{mr}. Varietal variability in simple correlation coefficients was high. There was no consistent correlation of surface to volume ratios with $r_a + r_s$, and Tr. There were no consistent trends in S_T/V_{CT} over the season.

Table 29. Mean exposed surface area to cellular volume ratios within the upper palisade tissue (S_U/V_{CU}), lower palisade tissue (S_L/V_{CL}), paraveinal tissue (S_{PV}/V_{CPV}), spongy mesophyll tissue (S_S/V_{CS}), and total exposed surface area to cellular volume within the leaves (S_T/V_{CT}) of four leaves per variety per year

Variety	Year	$\frac{S_U}{V_{CU}}$	$\frac{S_L}{V_{CL}}$	$\frac{S_{PV}}{V_{CPV}}$	$\frac{S_S}{V_{CS}}$	$\frac{S_T}{V_{CT}}$ [a]
Corsoy	1969	.35	.35	.17		.27
	1970	.20	.30	.12	.27	.22
	$\bar{x}$	.28	.33			.25
Amsoy	1969	.34	.35	.16		.26
	1970	.25	.32	.10	.29	.26
	$\bar{x}$	.30	.34			.26
Hawkeye	1969	.37	.36	.16		.28
	1970	.27	.28	.10	.28	.26
	$\bar{x}$	.32	.32			.27
Richland	1969	.38	.34	.11		.25
	1970	.22	.28	.12	.31	.25
	$\bar{x}$	.30	.31			.25
Provar	1970	.13	.26	.11	.28	.18
Lindarin	1970	.32	.33	.11	.34	.29
Means	1969	.36	.35	.15		.27
	1970	.23**	.30	.11	.30*	.24**
	$\bar{x}$	.30	.33			.26

[a] S_T/V_{CT} for 1969 was calculated from varietal means and not analyzed statistically.

*Significant varietal variation ($P<0.05$).

**Significant varietal variation ($P<0.01$).

Table 30. Simple correlation coefficients with exposed surface area to cellular volume ratios within various tissue layers between individual observations within and among varieties for two years and among varieties and years

Variety	Year	P_{320}					r_{mf}	
		S_U/V_{CU}	S_L/V_{CL}	S_{PV}/V_{CPV}	S_S/V_{CS}	S_T/V_{CT}[a]	S_U/V_{CU}	S_L/V_{CL}
Corsoy	1969	-.10	-.85	.12		.59	.38	.88
	1970	.63	.77	-.92	.59	.77	-.47	-.61
Amsoy	1969	.51	-.14	-.73		-.53	-.50	-.15
	1970	.63	-.93	.15	.26	.74	-.79	.95*
Hawkeye	1969	.09	-.02	-.49		.12	-.11	-.06
	1970	.24	.07	.55	.24	.56	.81	-.20
Richland	1969	.69	.41	.14		.75	.40	-.70
	1970	-.28	-.06	-.44	.03	-.40	.36	.32
Provar	1970	-.24	-.91	-.40	-.61	-.89	.37	.97*
Lindarin	1970	.49	-.16	.43	-.74	.06	.96*	.78
Among Varieties	1969	.12	.10	.10		.08	.28	-.16
	1970	-.18	.01	-.22	-.37	-.26	.15	-.05
Among Varieties and Years		-.21	.02	.27		.23	-.52**	-.53**

[a]V_{CT} for 1969 employed T_c in calculations rather than $V_{CU} + V_{CL} + V_{CPV} + V_{CS}$ as in 1970.

*Significant ($P<0.05$).

**Significant ($P<0.01$).

Table 30. (Continued)

Variety	Year	r_{mr}			DAY	$r_a + r_s$	Tr
		S_{PV}/V_{CPV}	S_S/V_{CS}	S_T/V_{CT}[a]	S_T/V_{CT}[a]	S_T/V_{CT}[a]	S_T/V_{CT}[a]
Corsoy	1969		.21	-.76	.69	.32	.96*
	1970	.87	-.70	-.60	.99**	-.16	.79
Amsoy	1969		.85	.70	-.88	.43	-.88
	1970	-.01	-.46	-.86	.68	.82	.65
Hawkeye	1969		.53	-.16	-.71	.55	-.13
	1970	-.70	.61	.44	-.77	-.75	.07
Richland	1969		.23	-.24	.54	-.74	.84
	1970	.76	-.04	.59	-.13	.05	-.35
Provar	1970	.54	.69	.88	-.89	.42	-.93
Lindarin	1970	.86	.01	.80	.72	.01	-.59
Among Varieties	1969		.26	-.04	.00	-.18	.14
	1970	.38	.29	.21	.11	.20	-.20
Among Varieties and Years			.71**	.45**	-.17	-.60**	-.11

V. DISCUSSION

A. Net Photosynthesis as Influenced by Test Conditions

Short and long term effects of environment on net photosynthesis are required for interpretation of results. Several important short term effects (while testing) on net photosynthesis are as follows: light quantity and quality, windspeed, carbon dioxide concentration, leaf temperature, and H_2O vapor pressure gradient between the leaf and the atmosphere.

Light-response curves were performed with two different light sources. An incandescent light source filtered by distilled water gave an indication that the photosynthetic process was saturated for most leaves at 6700 ft-c or 30 x 10^4 ergs$\cdot$sec$^{-1}\cdot$cm^{-2} (400-700 nm). In 1970, the same light source filtered by a 10^{-2} M $CuSO_4$ solution exhibited near saturation at 8150 ft-c or 26 x 10^4 ergs$\cdot$sec$^{-1}\cdot$cm^{-2} (400-700 nm). These light flux densities compare to about 49 x 10^4 ergs$\cdot$sec^{-1} (400-700 nm, 11,300 ft-c) for the sun. Various researchers (8, 13, 21, 57, 58, 59, 60) have reported light saturation for single soybean leaves and seedlings to be from 2,500 to 4,000 ft-c under varying experimental conditions and plant material. Data published by Kumura (59, 60) indicates that some leaves are not saturated at 40 kilolux (3,700 ft-c). Previous work (22) seemed to indicate that light saturation was a function of the photosynthetic rate, higher light saturation, and higher photosynthesis occurring later in the season. The high photosynthetic rates reported here substantiate the higher light flux density required for saturation of the

photosynthetic process. This is also confirmed by research with single leaves in a normal canopy and widely spaced plants without branches (5). Those results indicated that leaves of spaced plants possessed a much higher photosynthetic rate and were not light saturated at 15,000 ft-c. The normal field-grown canopy plants were light saturated at 10,000 ft-c. Thus, it is obvious that the light saturation level depends on the plant material and conditions of growth.

The effect of windspeed within the chamber is often tested by changing the flow rate of air into the chamber and then measuring its influence on net photosynthesis. This method is difficult to interpret because changing the flow rate also changes other environmental parameters. In 1970, a better test was performed by varying the chamber fan speed. This test indicated there was need for a fan for internal turbulence and that the fan employed gave optimum turbulence. Tests in 1968 and 1969 indicated optimum flow rates only. A windspeed of 138 cm/sec seemed to be sufficient for net photosynthesis of the soybean leaves tested.

Defining the level of carbon dioxide in the atmosphere is also essential for comparison of laboratory research work with field conditions. Earlier work (22) indicates that there is a very linear relationship between net CO_2 exchange and CO_2 level in the atmosphere for CO_2 levels in the range 0 to 300 ppm CO_2. (Normal atmosphere is approximately 320 ppm CO_2.) Net CO_2 exchange began to exhibit a curvilinear trend at 400 ppm CO_2. However, Brun and Cooper (13) have shown with growth chamber soybeans that net CO_2 exchange was linear with CO_2 concentration up to 700 ppm, or twice that of the atmosphere. The difference in

results of the two researchers is believed a consequence of difference in plant material.

To determine varietal potential it is important to know the response of that variety to temperature. In 1968, the optimum temperature for the leaves tested was 30 to 40°C. Optimum temperature was less than 35°C in 1969. These tests were not made under steady state conditions, and hence, one might expect the optimum to be lower if leaves were tested under steady state conditions for a significant length of time. Jeffers and Shibles (51) report an optimum air temperature of 25 to 30°C for net photosynthesis of an Amsoy soybean canopy. It has been reported (37) that soybean seedlings show little response of net photosynthesis to temperature from 15 to 30°C, although slight depression occurred in the range 26 to 30°C. Research data is insufficient to say whether varietal differences exist for optimum temperature for the net photosynthetic process. There is also some question as to what actually is the mean optimum temperature for soybeans.

Simple correlation coefficients between P_{320} and leaf temperature were -0.44 and -0.19 for 1969 and 1970. These negative correlation coefficients indicate that leaf temperature may have been too high both years, but especially in 1969.

Net photosynthetic response of the soybean leaves to vapor pressure deficit of the air (VPDA) has been little studied. In 1968, it was shown that net photosynthesis was unaffected by a VPDA of from 7.5 to 11.0 mm Hg. This test was with only one leaf, and the research leaves encountered mean VPDA of 16.5 mm Hg in 1969. Stevenson (95) has reported a positive

correlation between H_2O vapor pressure deficit of the air and leaf resistance to H_2O diffusion. His results would imply there is a possible relation between VPDA and net photosynthesis which is mediated via CO_2 diffusion resistance, since results of research herein indicate some stomatal control of net photosynthesis. It is considered possible that the high VPDL in 1969, 21 mm Hg, may have limited maximum expression of photosynthetic rate. Simple correlation coefficients of -0.53 and -0.27 between VPDL and P_{320} during 1969 and 1970 indicate that the VPDL may have been too high for optimum net photosynthesis in 1969 and 1970. Hawkeye was the only variety in 1969 that did not show a significant correlation of P_{320} with VPDL, but in 1970 Hawkeye and Richland were the only varieties that did show a significant correlation of P_{320} with VPDL.

B. Net Photosynthesis as Influenced by Leaf Age, Leaf Position, and Stage of Development

Optimum age of leaves for maximum photosynthesis was briefly investigated for four varieties in 1969. The leaves tested seemed to have a broad range of age for maximum net photosynthesis. In summary, maximum net photosynthesis does not occur exactly at full expansion, but from about two to six days after full expansion. This result agrees quite well with that of research with soybean variety Norin No. 2 (79), where maximum net photosynthesis was present from time of full leaf expansion to about one week after.

As discussed in the results, net photosynthesis, in general, increased during the season. The seasonal response was much greater in 1968 than in

1969 or 1970. This seasonal trend confirms earlier work (60, 61) that net photosynthesis for single leaves increases with the rise in position of the leaves on the main stem. Leaves from branches also have been reported to have lower photosynthetic rate than those on the main stem (60, 61). Position on the branches has also been shown important, the upper, more sunlit leaves having the highest photosynthetic rate (60). Isogenic (except for pubescence type) lines of Clark have shown a considerable increase in net photosynthesis (173-220%) between flowering and pod-filling stage (41). This type of response was reported earlier from the 1968 work (23); however, it was not evident in 1969 or 1970. Hansen (43) reports mean single leaf net photosynthetic rates for field-grown Corsoy soybeans (adjusted for light intensity, leaf temperature, and $r_a + r_s$) plants were 42, 34, 34, and 45 mg CO_2 dm^{-2} hr^{-1} for the four weeks during August of 1969. Increase in net photosynthesis during the season may be, in part, a result of demand for carbohydrate by the pods or some other control(s) of photosynthetic rate.

The confounding effects of plant age (stage of development), leaf age, and leaf position on the main stem make the interpretation of seasonal trends in leaf net photosynthesis very difficult. In 1968, P_{320} seemed to increase up to August 14-18, after which time the leaves became older with further testing. If it is assumed that leaf age was not responsible for the increase in P_{320} prior to August 14-18, then P_{320} trends, at least in part, may be due to leaf position and/or stage of plant development. After August 14-18, the leaf position on the main stem would not be a factor in P_{320} trends, but leaf age and stage of plant development may be.

C. Varietal Difference in Net Photosynthesis

Varietal differences in net photosynthesis of soybean leaves have been reported many times (12, 21, 22, 23, 24, 52, 78, 81). A relationship between several researcher's findings is presented in Figure 22. There is little or no relationship between the common varieties of the researchers and the relative photosynthetic rates obtained. There may be a slight positive correlation between the four common varieties of Dornhoff and Shibles (23) and Ojima et al. (81). If there were more varieties in common between these two groups of researchers a statistical test could be applied. It is obvious that there is no relation between the data of Curtis et al. (21) and Dornhoff and Shibles (23). Perhaps, the fact that Curtis et al. (21) used seedlings grown in growth chambers changed the photosynthetic potential of these varieties compared to those field-grown.

A study of verification of varietal differences in net photosynthesis under differing environments has been reported (78). It was found that the magnitude of P_{300} changed with environment, but varietal rankings remained the same. These results were not confirmed by the three year study of four varieties reported herein. It has been postulated that the lack of varietal differences in 1969, at least in part, were a result of environment during growth and not during testing. In essence, it appears that environment has affected the relative ranking of the varieties in this research, but possibly not that reported earlier (78).

The above discussion indicates there is a serious question as to the stability of net photosynthetic rate and the relative rates of the

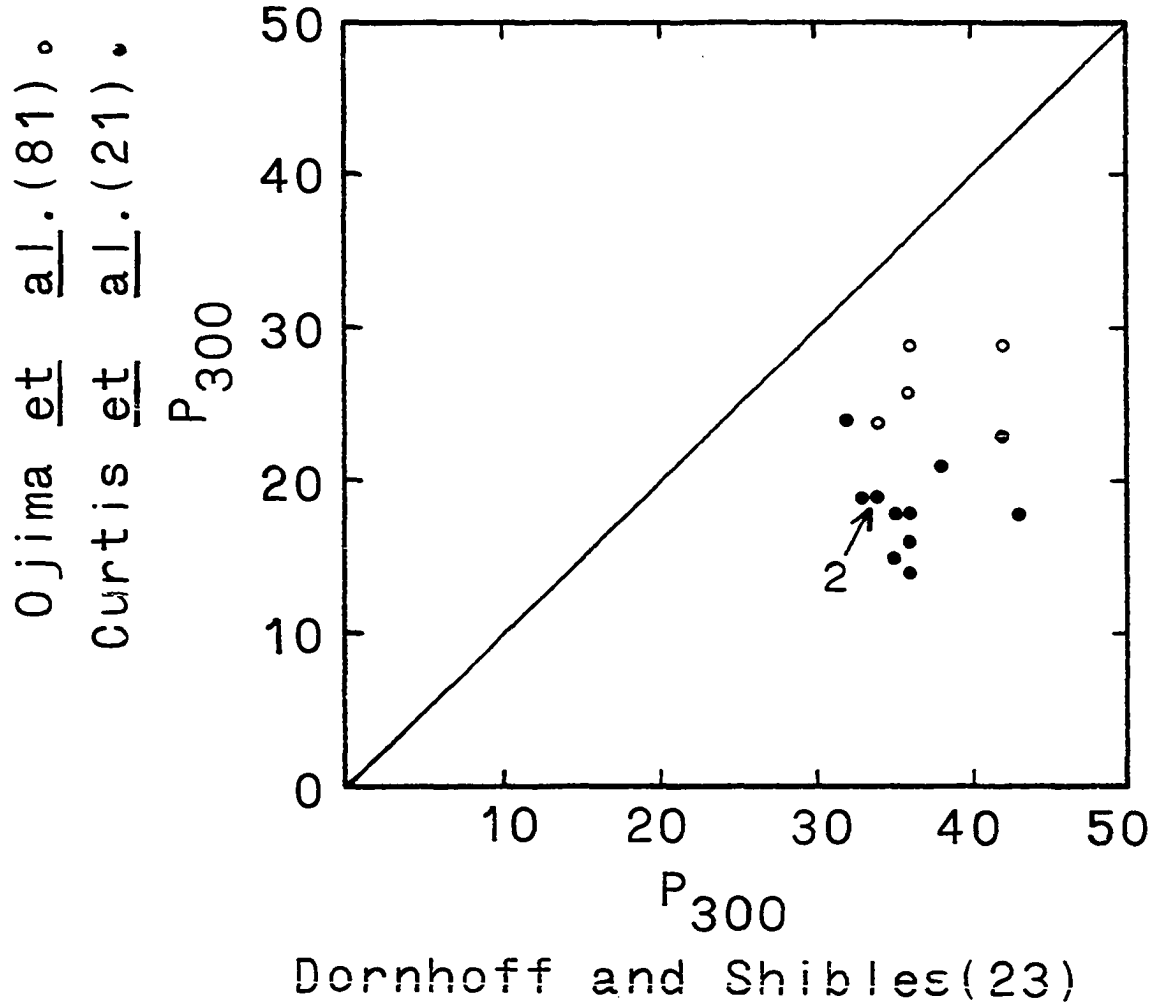

Figure 22. Net photosynthesis at 300 ppm CO_2. A comparison of the results of various researchers using the same varieties

varieties in different environments. It appears that further research needs to be done in this area.

D. Net Photosynthesis as Influenced by Respiration

Photorespiration (light respiration) in soybeans has been studied by several researchers (14, 16, 21, 22, 23, 35, 53, 67, 73). Reported literature indicates that soybeans evolve a significant amount of CO_2 in zero-CO_2 atmosphere and light, and they have a relatively high CO_2 compensation concentration (Γ). Research herein reported gives a Γ of 36-67 ppm for the twenty-two varieties examined during three years. Numerous researchers (16, 21, 35, 67) report values of Γ from 30 to 73 ppm CO_2. Soybean Γ is relatively high compared to that of maize, for example (Γ = 0 ppm), which seems to lack all indicators of photorespiration in significant magnitude to affect net photosynthesis.

Since it is possible that all methods of estimating photorespiration give underestimates of varying degree, it is difficult to assess the proportion of true photosynthesis which is utilized in light respiration. Research reported herein gives a minimal estimate of 29%--i.e., 29% of true photosynthesis is utilized in photorespiration, R_c (Table 6). CO_2 evolution in light and zero-CO_2 atmosphere (R_o) gives an estimate of 17% (7.6 x 100/(7.6 + 38.0)) for proportion of true photosynthesis utilized in photorespiration. Other research (21) indicates a possible 14% is attributable to photorespiration (R_o) in 36 varieties of soybeans. Comet soybeans exhibited 13% of true photosynthesis utilized in photorespiration, R_o (35). It is believed that the figure of 30% reported herein is

more accurate, since R_c is a better though minimal estimate of light respiration (89).

The relative magnitude of light respiration (R_o) compared to dark respiration of Comet soybeans is 1.5 = 6/4 (35). The ratio of photorespiration (R_o) to dark respiration may be estimated as 4.2 = 7.6/1.8 for this research (Figure 4 and Table 6). This value of 4.2 may be in error because the testing conditions were not the same for dark and light respiration. Dark respiration was determined at lower leaf temperature, lower humidity and at higher CO_2 (320 ppm). Dark respiration of Wayne in the field at 32°C was 6.8 mg CO_2 dm^{-2} hr^{-1} (52). This higher rate of dark respiration gives 6.8 x 100/(6.8 + 22.7) = 23% of true photosynthesis. The research reported herein gives 1.8 x 100/(1.8 + 38.0) = 4.5%. Japanese research indicates similar results (1.5 x 100/(1.5 + 22.5) = 6.2%) for the portion of dark respiration of true photosynthesis (79).

Varietal differences in various estimates of photorespiration have been reported (16, 21). Results in Table 6 indicate that R_o, R_c, and are sometimes significantly different among varieties. Generally, R_o and R_c are positively correlated with P_{320}. Positive correlations have also been reported for P_{320} with dark respiration (52, 78, 79). Thus, it seems that the higher the net photosynthetic rate, the higher the light and dark respiration. Perhaps a higher photosynthetic rate provides more substrate available for respiration or better yet, perhaps high net photosynthesis is accompanied generally be a more favorable metabolic state of the leaf (or plant). Thus, low light respiration may not be a desirable

character to select for in soybean lines, since photorespiration may not be an entirely wasteful process. CO_2 compensation concentration (Γ) seems negatively correlated to net photosynthesis (Table 8) and little differences in Γ for soybean varieties has been reported (16, 21). If variation exists in Γ, then perhaps this would be beneficial as a selection index for P_{320}, but variation evidently does not exist in significant magnitude (16).

In essence, there exists not only a question of what is the net photosynthetic and light respiratory rates of different soybean genotypes, but also the ratio of light respiration to net (or true) photosynthesis.

E. Net Photosynthesis as Influenced by Anatomy and Diffusion Resistances

1. Leaf stomata

As pointed out in the literature section, it is often said that photosynthesis of crop plants is chiefly limited by carbon dioxide. This limitation usually is thought a consequence of a physical resistance to diffusion of CO_2 from the atmosphere above the canopy to the site of fixation in the leaf. Resistance due to the atmosphere (external to the leaf), r_a, generally is found quite low in magnitude relative to other resistances. r_a was about 1.0 sec/cm (19% of $\sum r_r$) for the soybean leaves tested in this research. As mentioned previously, this estimate may be high. An r_a of 1.1 sec/cm was reported for Lee soybeans (28). This r_a of 1.1 was 12% of the total resistance to CO_2 diffusion ($\sum r_o = 9.3$). Stevenson (95) reports a value of 0.77 sec/cm for soybeans using the "Linacre" technique of measuring r_a (involves the measurement of leaf temperature changes after the leaf is shaded) of leaves in the field. He

also gives a value of 0.06 sec/cm which was derived from an energy balance equation. Stevenson concludes that an r_a = 0.77 sec/cm for H_2O diffusion is unreasonably large. This would be an r_a value of 0.77 (1.56) = 1.2 for CO_2 diffusion. In view of the fact that Stevenson's r_a for soybeans is believed to be less than 1.2 sec/cm and the r_a reported in Table 9 are probably too large, it may be postulated that r_a is less than 1.0 sec/cm, or less than 19% of the total assumed resistance to CO_2.

The next physical resistance encountered by CO_2 is r_s or resistance to diffusion of CO_2 from the external surface of the leaf to the surface of the mesophyll walls. r_s includes resistance of the epidermal surfaces of the leaves. Under normal conditions the resistance of the cuticle is probably quite high, hence most CO_2 enters via the stomata.

Stomatal resistance, r_s, is generally larger than r_a. The mean stomatal resistance was 1.4 sec/cm for the three years. This compares to an r_s of 2.7 for Lee (28). In this research, r_s accounts for 27% of the total resistance, $\sum r_r$, and $r_a + r_s$ account for 46%. For Lee (28) r_s is 29% of $\sum r_o$, and $r_a + r_s$ account for 41% of $\sum r_o$. Thus, 40-50% of the resistance to net photosynthesis is probably a result of physical resistances to CO_2 diffusion of the atmosphere, stomata apertures and frequencies, and intercellular space within the leaves. Boyer (9) found $r_a + r_s$ of 2 sec/cm and a $\sum r_r$ of 5 sec/cm for Harosoy soybeans. His estimate of $r_a + r_s$ is 40% of $\sum r_r$, and hence, it closely approximates the results of this research.

Stomatal diffusive resistances were highly correlated with net photosynthesis of soybeans all three years. Varietal differences in r_s and

$r_a + r_s$ also indicate r_s is a possible cause for varietal differences in P_{320}. Thus, physical resistance of stomata accounts for some of the environmental and genetic variability in P_{320} under these conditions of testing. As indicated in the results, $r_a + r_s$ accounted for 22 and 13% of the variation in P_{320} in 1969 and 1970. There was also a large amount of variability in P_{320} accounted for by $r_a + r_s$ and r_{mf} combined (Table 12). In 1969, $r_a + r_s$ seemed to be regulating P_{320} more than r_{mf}, while in 1970, the reverse was true.

Observations of stomatal anatomy have, in general, confirmed the above conclusion of stomatal control of photosynthesis. Stomatal apertures and densities were often correlated among varieties to photosynthetic rate; the highest correlations were for 1969. Laminar plus stomatal resistance to CO_2 diffusion generally was negatively correlated with stomatal apertures and densities. The positive correlation of P_{320} with stomatal anatomy, the negative correlation of $r_a + r_s$ with stomatal anatomy, and the negative correlation of P_{320} with $r_a + r_s$, along with the partitioning of sums of squares by multiple linear regression suggest a cause and effect relationship for stomatal control of net photosynthesis.

Statistically significant varietal differences in stomatal apertures were present only for width of stomata on the adaxial surface (W_{ad}). There were varietal differences in density of stomata (N_{ad} and N_{ab}), but they were not related closely to net photosynthetic rate. There seems to be only limited evidence of stomatal apertures and densities partially limiting or controlling P_{320} among varieties. However, there is a trend for the high photosynthesizing varieties to exhibit larger stomatal apertures (primarily W_{ad} and L_{ad}) than the low photosynthesizing varieties.

2. Leaf mesophyll

The remaining resistance encountered by CO_2 is mesophyll resistance, which is resistance to CO_2 transport from the mesophyll cell wall to the site of fixation. To date, there is no way of completely separating the physical and chemical resistances of which r_m is composed. It is often hypothesized that r_m is related to the mesophyll anatomy of the leaves.

It seems obvious that, if there is a cause and effect relationship between DT, TH, and P_{320}, the thicker the leaf the more photosynthetic machinery there is present. The question that remains unanswered is what part(s) of this machinery, besides stomata control, regulates photosynthetic rate. Dimensions of the leaf mesophyll for several varieties were examined in attempt to further partition the control of net photosynthesis.

Partial correlation coefficients for various anatomical features were calculated in attempt to elucidate the mechanism of mesophyll control of photosynthesis (Table 31). Simple correlation coefficients indicated a positive relationship between P_{320} and thickness of each tissue layer. However, partial correlation coefficients indicate that leaf thickness, T_c, was correlated both years to the thickness of the various tissue layers except the lower palisade parenchyma, L_L. The lack of a positive correlation of L_L with T_c suggests that variation in L_L is not related to variation in T_c.

Since total internal exposed surface area, S_T, was not significantly different among varieties, S_T would seem not strongly related to varietal differences in net photosynthesis. However, simple correlation coefficients indicate that S_T is positively related to P_{320} (especially for

Table 31. Partial correlation coefficients of net photosynthesis, density-thickness, thickness, and diffusion resistances with various anatomical characters of individual observations among varieties. 16 observations in 1969 and 24 in 1970

Correlation	Year	r
T_c, $L_U \cdot L_L$, L_S	1969	0.38
T_c, $L_L \cdot L_U$, L_S	1969	-0.20
T_c, $L_S \cdot L_U$, L_L	1969	0.58
T_c, $L_U \cdot L_L$, L_{PV}, L_S	1970	0.45
T_c, $L_L \cdot L_U$, L_{PV}, L_S	1970	-0.07
T_c, $L_{PV} \cdot L_U$, L_L, L_S	1970	0.47
T_c, $L_S \cdot L_U$, L_L, L_{PV}	1970	0.15
T_c, $S_T \cdot V_T$, V_{CT}	1969	0.02
T_c, $V_T \cdot S_T$, V_{CT}	1969	0.98
T_c, $V_{CT} \cdot S_T$, V_T	1969	1.00
TH, $S_T \cdot V_T$, V_{CT}	1970	-0.37
TH, $V_T \cdot S_T$, V_{CT}	1970	0.72
TH, $V_{CT} \cdot S_T$, V_T	1970	0.73
DT, $S_T \cdot V_T$, V_{CT}	1969 1970	0.68 -0.52
DT, $V_T \cdot S_T$, V_{CT}	1969 1970	0.71 0.56
DT, $V_{CT} \cdot S_T$, V_T	1969 1970	0.64 0.77
P_{320}, $S_T \cdot V_T$, V_{CT}	1969 1970	0.46 0.67

Table 31. (Continued)

Correlation	Year	r
P_{320}, $V_T \cdot S_T$, V_{CT}	1969	0.43
	1970	-0.46
P_{320}, $V_{CT} \cdot S_T$, V_T	1969	0.01
	1970	0.74
$r_a + r_s$, $S_T \cdot V_T$, V_{CT}	1969	-0.30
	1970	-0.14
$r_a + r_s$, $V_T \cdot S_T$, V_{CT}	1969	-0.12
	1970	0.11
$r_a + r_s$, $V_{CT} \cdot S_T$, V_T	1969	0.19
	1970	-0.20
r_{mr}, $S_T \cdot V_T$, V_{CT}	1969	-0.22
	1970	0.38
r_{mr}, $V_T \cdot S_T$, V_{CT}	1969	-0.55
	1970	-0.53
r_{mr}, $V_{CT} \cdot S_T$, V_T	1969	-0.18
	1970	-0.58

1969). Partial correlation coefficients indicate that S_T is positively related to P_{320}, holding V_T and V_{CT} constant, which implies a relationship between S_T and P_{320} independent of V_T and V_{CT}. More exposed internal surface area would seem beneficial from the standpoint of more CO_2 absorptive surface area. Thus, mesophyll resistance should be negatively related to S_T. Both years, r_{mr}, exhibited a negative simple correlation to S_T. However, partial r's, holding V_T and V_{CT} constant indicate a negative correlation in 1969 of r_{mr} with S_T and a positive relation in

1970. Hence, it seems photosynthesis is not strongly related to internal surface area within the leaf mesophyll as a result of r_{mf}. $r_a + r_s$ exhibited a negative relationship with S_T both years (Table 31), though the correlation is small. In summary, S_T evidently is related to P_{320} but the mechanism of this relationship is at present unclear.

Varietal differences in net photosynthesis seem related to volume of intercellular space (V_T). Significant varietal differences in V_T were present in 1970. In 1969, the high photosynthesizing varieties were also higher in relative intercellular space, though varietal differences were not statistically significant. Variation in P_{320} within varieties was positively related to V_T in 1969 and negatively related to V_T in 1970 holding S_T and V_{CT} constant (Table 31). Photosynthesis would be expected to be related to volume of intercellular space via $r_a + r_s$, but $r_a + r_s$ was not significantly correlated to volume of intercellular space. However, holding S_T and V_{CT} constant, r_{mf} was strongly negatively related to V_T in both 1969 and 1970. It is not known for certain why r_{mf} is negatively correlated to volume of intercellular space. If the intercellular spaces are essentially saturated with H_2O vapor, then by method of calculation, r_{mf} could include part of the resistance to diffusion of CO_2 within the intercellular spaces. However, it seems that the resistance of the intercellular space would be small compared to other components of r_{mf}--the physical and chemical resistances within the cells.

There were no significant varietal differences in cellular volume, but in general, the high photosynthesizing varieties exhibited higher

V_{CT} then the low photosynthesizing varieties. Thus, it seems that part of the varietal differences in P_{320} may be related to cellular volume. For 1970, the variation among varieties of P_{320} seems related to V_{CT} holding S_T and V_T constant; while in 1969, there was no relationship between P_{320} and V_{CT}. Evidently, the relationship between P_{320} and V_{CT} is related to r_{mf}, since r_{mf} is negatively correlated to V_{CT}, holding S_T and V_T constant.

The ratio of exposed cellular surface area to cellular volume was not related to P_{320}. The lack of an S_U/V_{CU} to P_{320} and S_L/V_{CL} to P_{320} relationship was expected because diameters of the palisade cells were not related to P_{320}. The smaller the cells are the higher the ratio of cell surface to cell volume. The lack of a correlation of P_{320} with S_T/V_{CT} implies that there is no advantage of smaller cells with more relative surface area. The relationship of S_T/V_{CT} with P_{320} has been used to partially distinguish between physical diffusion and other biochemical factors limiting P_{320} within the leaf mesophyll (28, 65). Since soybeans exhibit no relationship between P_{320} and S_T/V_{CT}, it might be postulated that biochemical factors are chiefly responsible for mesophyll control of net photosynthesis. However, it is believed that the S_T/V_{CT} to P_{320} relationship does not necessarily distinguish the physical and biochemical factors controlling P_{320}. Mesophyll control of P_{320} is probably a combination of physical diffusion resistances and biochemical factors (many of which were discussed in the Literature Review).

F. Selecting for Net Photosynthesis

A recent study of F_1 and F_2 generations of two crosses of soybeans indicates that inheritance of net photosynthetic rate in these genotypes was of a quantitative nature (80). Without exception, the F_2 generations exhibited a normal distribution for net photosynthetic rate. These results are not surprising, because net photosynthesis is thought to be regulated by several, or even many, processes.

As discussed in the results section, net photosynthesis was positively correlated to DT (leaf dry weight/leaf area). The simple correlation coefficients among varieties were greater than 0.40 all three years (Table 16), and the genetic correlation coefficients were greater than 0.90 (Table 18). Because of the high correlation between the two variables, DT has been postulated a possible selection index for photosynthetic efficiency (23). Leaf thickness also was positively correlated to P_{320} in 1969 and 1970 ($r = 0.43$ in 1969 and 0.58 in 1970). This parameter would be easier to measure than DT. It is believed, however, that the P_{320}-TH relationship should be tested thoroughly in the field, because differences in leaf water potential may affect the correlation.

Ojima and Kawashima (78) showed a high positive correlation of DT with P_{320} in the first and fourth leaf from the bottom in 1964. The correlation was also present for the tenth and twelfth leaves, but was smaller and not significant at the 5% level. The same type of relation was exhibited in their 1966 data. In 1967, the correlation of P_{300} of the fourth and sixth leaves with DT also was highly significant. The present

research seems to confirm their results. But, there seem exceptions to the relationship under low fertility conditions (78) and with F_2 populations (80), where the simple correlations of DT-P_{320} were not significant and they were small in magnitude. A more consistent correlation of TH-P_{300} was reported for F_2's than DT-P_{300} (80).

It is believed that selectability for P_{320} would be similar to selectability for yield; it depends on the environment strongly. Selection should be performed in the same ecological environment as the selected plants are to be grown. Because of the yearly variation in environment and its influence on P_{320}, it is believed that selection should be made using a large number of plants during several years. At present, DT and TH are the best indexes of leaf photosynthesis.

VI. SUMMARY

Net photosynthesis of soybean leaves exhibited varietal differences in 1968 and 1970, but not in 1969. It is believed that the differences among years in net photosynthesis is a result of different experimental conditions and different environments under which the plants were grown. The relative rankings of photosynthetic rates of varieties in common among different researchers does not agree. Evidently, the photosynthetic rate of different soybean varieties is influenced strongly by environment during growth and hence, environmental variation may present a serious problem in selection for photosynthetic efficiency among lines of soybeans.

Net photosynthesis of soybeans was positively related to light respiration. Light respiration accounted for an average of 29% of the apparent true photosynthesis. CO_2 compensation concentration was negatively related to net photosynthesis, but varietal differences in CO_2 compensation concentration were small.

Laminar and stomatal resistances to CO_2 diffusion accounted for 22 and 13% of the variability in photosynthesis in 1969 and 1970. In general, stomatal apertures and densities were positively related to photosynthesis and negatively related to stomatal resistance to diffusion of CO_2.

Mesophyll resistance accounts for 15 and 53% of the variability in photosynthesis in 1969 and 1970.

Simple and genetic correlation coefficients indicated a strong relationship between density-thickness, thickness, and net photosynthesis. All experimental evidence indicated that density-thickness was primarily

measuring variation in leaf thickness. Leaf thickness and density-thickness are suggested possible selection indexes for leaf photosynthesis.

Thickness of the various tissues within the leaf (upper palisade parenchyma, paraveinal mesophyll, and spongy parenchyma) except thickness of lower palisade parenchyma layer were positively related to leaf thickness. In 1969, net photosynthesis was positively related to internal exposed cell surface area and volume of intercellular space. In 1970, photosynthesis was positively related to exposed surface area and cellular volume and negatively related to volume of intercellular space. There was no significant and consistent relationship between net photosynthesis and ratio of exposed surface area to cellular volume.

Seasonal trends in net photosynthesis were not reproducible among years. The increase in net photosynthesis was postulated a result of leaf age, insertion level of leaf on the main stem, and/or physiological stage of development of the plant. In general, the seasonal trends of other variables could be predicted from their relationship to net photothesis.

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IX. APPENDIX

Table 32. Statistics (degrees of freedom for varieties and error, df; ratio of variety to error variance, F; standard error of difference between treatment means, $s_{\bar{d}}$; least significant difference between varietal means (P 0.05), LSD(.05); and coefficient of variability, CV)

Variable	Year	df	F	$s_{\bar{d}}$	LSD(.05)	CV
VPDA	1969	3,69	1.8	0.73	1.5	15
	1970	5,125	9.4**	0.31	0.61	16
VPDL	1969	3,69	0.7	1.02	2.0	16
	1970	5,125	9.2**	0.31	0.61	16
RH-IN	1969	3,69	0.2	0.75	1.5	10
	1970	5,125	0.5	0.48	0.95	4
RH-OUT	1969	3,69	4.2**	1.33	2.7	10
	1970	5,125	11.3**	1.1	0.22	5
LT	1968	19,266	1.5+	0.50	1.0	4
	1969	3,69	0.3	0.49	0.98	5
	1970	5,125	2.4*	0.13	0.26	2
AT	1969	3,69	0.3	0.41	0.82	5
	1970	5,125	1.5	0.13	0.26	2
P_{300}	1968	19,266	6.3**	2.0	3.8	15
P_{320}	1969	3,69	1.8	1.8	3.6	17
	1970	5,125	11.7**	1.5	3.0	14
P_{wt}	1968	19,266	1.7*	4.5	8.8	13
	1969	3,69	0.5	5.5	11	16
	1970	5,125	3.0*	5.6	11	13
R_o	1968	19,266	1.5+	0.6	1.2	30
	1969	3,69	2.3+	0.52	1.0	20
	1970	5,125	3.3**	0.34	0.67	14
R_c	1968	19,117	1.0	—	—	56
	1970	5,125	4.1**	0.48	0.95	15
P_i	1968	19,117	2.1**	—	—	22
	1970	5,125	12.1**	1.7	3.4	12
R_c/P_i	1968	19,117	0.6	—	—	34
	1968	19,266	1.0	3.4	6.7	24
	1969	3,69	0.3	3.5	7.0	18
	1970	5,125	3.0*	2.0	4.0	13
Tr	1968	19,117	2.1*	—	—	19
	1969	3,69	2.0	0.15	0.30	13
	1970	5,125	5.1**	0.16	0.32	15

+Significant varietal variation (P<0.10).

*Significant varietal variation (P<0.05).

**Significant varietal variation (P<0.01).

Table 32. (Continued)

Variable	Year	df	F	$s_{\bar{d}}$	LSD(.05)	CV
P/Tr	1969	3,69	0.2	0.041	0.082	16
	1970	5,125	2.4*	0.042	0.083	14
S	1968	19,266	6.2**	0.01	0.015	15
	1969	3,69	2.4+	0.0064	0.013	16
	1970	5,125	11.6**	0.0053	0.010	13
$\sum r_o$	1969	3,69	0.7	0.73	1.5	37
	1970	5,125	10.5**	0.28	0.55	17
$\sum r_f$	1968	19,266	3.9**	0.4	0.78	20
	1969	3,69	0.9	0.44	0.88	28
	1970	5,125	10.7**	0.21	0.42	15
$r_a + r_s$	1968	19,117	2.7**	—	—	24
	1969	3,69	0.8	0.23	0.46	27
	1970	5,125	4.6**	0.098	0.19	32
r_a	1969	3,69	0.8	0.038	0.076	14
	1970	5,125	9.1**	0.039	0.077	16
r_s	1968	19,117	2.5**	—	—	36
	1969	3,69	0.9	0.21	0.42	34
r_{mo}	1969	3,69	0.6	0.55	1.1	50
r_{mf}	1968	19,117	2.0*	—	—	33
	1969	3,69	1.2	0.26	0.52	38
	1970	5,125	12.3**	0.16	0.32	15
DT	1968	19,266	7.2**	0.02	0.04	13
	1969	3,69	8.9**	0.0078	0.016	9
	1970	5,125	10.9**	0.0078	0.015	11
DT-V	1969	3,69	3.4*	0.012	0.024	10
TH	1969	3,69	18.4**	1.9	3.8	8
	1970	5,125	30.9**	1.3	2.6	7
Area	1968	19,266	11.6**	0.035	0.078	14
	1969	3,69	14.3**	0.028	0.056	14
	1970	5,125	26.2**	0.028	0.055	13
W_{ad}	1969	3,9	3.1+	1.6	3.6	60
	1970	5,15	0.6	1.3	2.8	68
W_{ab}	1969	3,9	0.8	1.6	3.6	31
	1970	5,15	2.1	1.1	2.3	33
L_{ad}	1969	3,9	2.0	3.0	6.8	42
	1970	5,15	1.0	1.3	2.8	14
L_{ab}	1969	3,9	0.9	0.92	2.1	11
	1970	5,15	1.3	1.1	2.3	14
N_{ad}	1969	3,9	7.4**	31	70	24
	1970	5,15	1.6+	33	70	17
N_{ab}	1969	3,9	3.8	36	81	13
	1970	5,15	10.1**	42	90	11

Table 32. (Continued)

Variable	Year	df	F	$s_{\bar{d}}$	LSD(.05)	CV
T_m	1969	3,9	4.0*	16	36	11
	1970	5,15	3.4*	9.9	21	8
T_c	1969	3,9	8.9**	14	32	12
	1970	5,15	3.5*	31	66	17
L_U	1969	3,9	1.9	5.9	13	18
	1970	5,15	0.6	4.0	8.5	16
L_L	1969	3,9	1.6	4.2	9.5	15
	1970	5,15	0.4	5.3	11	20
L_{PV}	1970	5,15	1.7	2.3	4.9	27
$L_{PV} + L_S$	1969	3,9	1.7	7.1	16	19
L_S	1970	5,15	3.4*	6.6	14	27
D_U	1969	3,9	3.6+	0.54	1.2	9
	1970	5,15	1.2	0.54	1.2	10
D_L	1969	3,9	0.9	0.66	1.5	10
	1970	5,15	2.5+	0.50	1.1	8
S_U	1969	3,9	0.9	1.7	3.8	24
	1970	5,15	2.6+	0.92	2.0	22
S_L	1969	3,9	0.8	1.1	2.5	23
	1970	5,15	0.8	1.1	2.3	23
S_{PV}	1970	5,15	2.2	0.16	0.34	26
$S_{PV} + S_S$	1969	3,9	4.8*	0.99	2.2	27
S_S	1970	5,15	3.6*	0.99	2.1	34
S_T	1969	3,9	1.9	3.2	7.2	23
	1970	5,15	2.0	2.5	5.3	21
S_U/S_T	1969	3,9	4.1*	0.026	0.059	8
	1970	5,15	2.6+	0.028	0.060	11
S_L/S_T	1969	3,9	1.4	0.022	0.050	10
	1970	5,15	4.5*	0.028	0.060	10
S_{PV}/S_T	1970	5,15	3.0*	0.012	0.026	34
$(S_S + S_{PV})/S_T$	1969	3,9	2.4	0.038	0.086	23
S_S/S_T	1970	5,15	3.7*	0.036	0.077	22
S_U/S_E	1969	3,9	0.9	0.70	1.6	24
	1970	5,15	2.6+	0.40	0.85	24
S_L/S_E	1969	3,9	0.8	0.48	1.1	24
	1970	5,15	0.8	0.44	0.94	23
S_{PV}/S_E	1970	5,15	2.2	0.067	0.14	26
$(S_S + S_{PV})/S_E$	1969	3,9	4.8*	0.41	0.93	28
S_S/S_E	1970	5,15	3.6*	0.40	0.85	34
S_T/S_E	1969	3,9	1.9	1.3	2.9	20
	1970	5,15	2.0	0.99	2.1	19
V_U	1969	3,9	0.9	4.3	9.7	22
	1970	5,15	5.5**	2.1	4.5	17

Table 32. (Continued)

Variable	Year	df	F	$s_{\bar{d}}$	LSD(.05)	CV
V_L	1969	3,9	0.7	5.8	13	30
	1970	5,15	1.1	3.4	7.2	23
V_{PV}	1970	5,15	2.1	1.3	2.8	28
$V_{PV} + V_S$	1969	3,9	0.8	4.4	10	22
V_S	1970	5,15	2.6+	5.0	11	26
V_T	1969	3,9	0.9	12	27	21
	1970	5,15	3.2*	8.5	18	17
V_U/V_{EU}	1969	3,9	0.3	0.061	0.14	18
	1970	5,15	4.9**	0.049	0.10	17
V_L/V_{EL}	1969	3,9	0.1	0.092	0.21	22
	1970	5,15	1.1	0.067	0.14	19
V_{PV}/V_{EPV}	1970	5,15	0.9	0.050	0.11	16
$(V_{PV} + V_S)/(V_{EPV} + V_{ES})$	1969	3,9	1.2	0.022	0.050	7
V_S/V_{ES}	1970	5,15	0.9	0.050	0.11	11
V_T/V_{ET}	1969	3,9	1.0	0.058	0.13	21
	1970	5,15	4.1*	0.030	0.064	17
V_{CU}	1969	3,9	1.3	5.5	12	27
	1970	5,15	1.6	4.3	9.2	23
V_{CL}	1969	3,9	0.2	4.4	10	31
	1970	5,15	0.6	5.2	11	32
V_{CPV}	1970	5,15	1.3	1.8	3.8	31
$V_{CPV} + V_{CS}$	1969	3,9	2.7	4.8	11	19
V_{CS}	1970	5,15	3.2*	3.8	8.1	38
V_{CT}	1969[a]	3,9	8.3**	14	32	24
	1970	5,15	0.9	11	23	23
S_U/V_{CU}	1969	3,9	0.8	0.028	0.063	11
	1970	5,15	11.9**	0.026	0.055	16
S_L/V_{CL}	1969	3,9	0.2	0.035	0.079	14
	1970	5,15	1.3	0.031	0.066	15
S_{PV}/V_{CPV}	1970	5,15	1.0	0.011	0.023	15
$(S_{PV} + S_S)/(V_{CPV} + V_{CS})$	1969	3,9	1.5	0.031	0.070	29
S_S/V_{CS}	1970	5,15	3.2*	0.021	0.045	10
S_T/V_{CT}	1969[a]	3,9	0.7	0.040	0.090	21
	1970	5,15	12.6**	0.015	0.032	9

[a] T_c used in calculation.